SOCIAL DECISION-MAKING IN A GROUP LIVING CICHLID FISH

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ABSTRACT

For my doctoral research I examined social decision-making in a cooperatively breeding cichlid fish, *Neolamprologus pulcher* with a focus on affiliation and aggression. I investigated the role that the nonapeptide hormone, isotocin, plays in modulating social decisions in these contexts. I show that *N. pulcher* males prefer to join larger groups regardless of the rank at which they will join, whereas females prefer larger groups only when they can join a group in a high rank (Chapter 2). I examined decision-making during resource contests in (Chapter 3) and found that *N. pulcher* are sensitive to the size of their opponents, making fighting decisions depending on their opponents' body size. I also found that smaller *N. pulcher* are more motivated to persist within contests, showing a shorter latency to resume fighting following interruption (Chapter 4). In Chapters 5 and 6, I explored the role of isotocin (the teleost fish homologue of oxytocin) in regulating social behaviour. I discovered that an increase in isotocin increased responsiveness to social information. Fish treated with isotocin were more sensitive to their opponent's size in contests and were more submissive to dominant individuals within their social group (Chapter 5). Unexpectedly, I found that exogenous isotocin reduced sociality in *N. pulcher,* and that an isotocin receptor antagonist increased it (Chapter 6). These results suggest that the relationship between isotocin and social behaviour is both complex and context specific. In my final data chapter, I used social network analysis to explore the role of dominance interactions in determining the structure of *N. pulcher* social groups. I found that *N. pulcher* dominance hierarchies are highly linear, but that dominance interactions are not predicted by sex or body size asymmetry (Chapter 7). I found that conflict within *N. pulcher* social groups is greatest at the top of the dominance hierarchy.

Taken together the results of my thesis helps to elucidate the behavioural and hormonal basis of social decision-making in a cooperatively breeding vertebrate and help to illuminate the evolution of social behaviour.

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DECLARATION OF ACEDEMIC ACHIEVEMENT

This thesis is organized in sandwich format as approved by McMaster University. It consists of eight chapters. **Chapter 1** provides a general introduction to social decision-making, aggression, sociality, social networks and the nonapeptide hormone oxytocin. **Chapters 2, 3, 4, 5 and 7** are published papers. **Chapter 6** is a manuscript currently under review. **Chapter 8** provides a synthetic discussion of the results of **Chapters 2-7** and addresses some of the questions raised including suggestions for future research.

Chapter 1 – General introduction

Author: Adam R. Reddon

Chapter 2 - Sex differences in group-joining decisions in social fish

Authors: Adam R. Reddon, Daniel Balk and Sigal Balshine

Publication: Animal Behaviour, 2011, 82:229-234

Comments: ARR conceived of the study and designed the experiments. ARR collected the data. DB scored behaviours from video recordings. ARR conducted the analysis and wrote the first draft of the manuscript. ARR and SB revised the manuscript. Reprinted with permission.

Chapter 3 - Rules of engagement for resource contests in a social fish

Authors: Adam R. Reddon, Mathew R. Voisin, Nandini Menon, Susan E. Marsh- Rollo, Marian Y. L. Wong and Sigal Balshine

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Chapter 4 - Probing aggressive motivation during territorial contests in a group-living cichlid fish

Authors: Adam R. Reddon, Daniel Balk and Sigal Balshine

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Chapter 5 - Effects of isotocin on social responses in a cooperatively breeding fish

Authors: Adam R. Reddon, Constance M. O'Connor, Susan E. Marsh-Rollo and Sigal Balshine

Comments: ARR and SB conceived of the study and designed the experiments. ARR, CMO and SEM-R collected the data. ARR conducted the analysis. ARR wrote the first draft of the manuscript. ARR, CMO and SB revised the manuscript. SEM-R produced the final figures. Reprinted with permission.

Chapter 6 - Isotocin and sociality in a cooperatively breeding cichlid fish

Authors: Adam R. Reddon, Mathew R. Voisin, Constance M. O'Connor and Sigal Balshine

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Chapter 7 - Network structure is related to social conflict in a cooperatively breeding fish

Authors: Cody J. Dey, Adam R. Reddon, Constance M. O'Connor and Sigal Balshine

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Chapter 8 – General discussion

Author: Adam R. Reddon

Chapter 1: General introduction

Adam R. Reddon

Perhaps because of the exceptionally social nature of our own species, social behaviour has long held particular fascination for animal behaviourists, and evolutionary ecologists (Alexander 1974, Wilson 1975; Székely et al. 2010). Attempts to understand social behaviour have generated some of the most important and enduring questions in behavioural biology and have stimulated some of the most significant discoveries in our understanding of how and why behaviour has evolved (e.g., Hamilton 1964a, b; Trivers 1971, 1974; Wilson 1975, Maynard-Smith & Parker 1976). Despite the long-standing interest and the intensity of research effort dedicated towards understanding social interactions, key questions about the evolution of social systems remain unanswered (Trivers 1971, Drews 1993; Dugatkin 1997; Bourke 1997; Krause & Ruxton 2002; Reader 2003; Galef & Laland 2005; Clutton-Brock 2009; Hatchwell 2009; Earley & Dugatkin 2010; Székely et al. 2010). For example, what factors drive some animals to spend their lives in close association with conspecifics while others live primarily solitary lives? Why do some species but not others exhibit complex social behaviours such as social learning, cooperation, the formation of dominance hierarchies, and in some exceptional cases, even a division of labour among group members? How do these complex social behaviours evolve? In order to answer these important questions, it is crucial that we understand social behaviour at both the functional level (i.e., what factors select for social behaviour in the first place; Krebs & Davies 1997) and the proximate level (i.e., what neural and physiological mechanisms support social behaviour; Goodson 2005, 2008; Soares et al. 2010). In this thesis, I endeavor to elucidate both the how and why of social behaviour using *Neolamprologus pulcher*, a highly social cichlid fish and an emerging model system for the integrative study of social system evolution. In this general introduction, I will provide the background that lays the framework for the data chapters that follow.

1. Sociality

It has been argued that complex social systems and sophisticated social behaviours have emerged as a result of an accumulation and combination of simpler discrete behavioural modules or building blocks (Soares et al. 2010). For example, species that live in groups must have first evolved a tolerance for conspecifics, followed by the social motivation that would draw individuals together (Soares et al. 2010). Sociality, or the tendency for individuals to cluster together in space and time (Alexander 1974), is one of the most fundamental components of social behaviour, setting the stage for more complex social interactions (Soares et al.2010; Goodson 2013). Only once groups have formed, can more complex social organization, like cooperative breeding, evolve (Earley & Dugatkin 2010). The degree of sociality varies among individuals, populations, and species (Krause & Ruxton 2002; Cote et al.2010), and the extent to which animals form groups will depend on the tradeoff between the advantages and the disadvantages of social living at these levels of biological organization (Alexander 1974). Living in a group can provide substantial advantages such as the abatement of predation through predator dilution, predator confusion, and shared vigilance (Hamilton 1971; Alexander 1974; Wilson 1975; Krause & Ruxton 2002, 2010; Earley & Dugatkin 2010). Grouping can also benefit individuals by increasing mating opportunities, improving foraging success, reducing movement costs, facilitating cooperation and promoting learning from social fellows (reviewed in Krause & Ruxton 2002). In contrast, group living can also carry substantial costs including increased conspicuousness to predators (Cresswell 1994), competition for mates, food, or other resources (Janson & Goldsmith 1995) and the spread of disease or parasites (Brown & Brown 1986). Regardless of the particular trade-offs, sociality represents a key building block of complex social behaviour and in order to understand how complex social systems evolve it is essential that we understand the factors, both proximate and ultimate, that drive variation in sociality both within and across species.

2. *Social partner assessment and decision-making*

Social partner assessment, or the ability to gather information about another individual with whom there will be likely be an interaction, is an important ability for highly social animals (Elwood & Arnott 2012; Riechert 2013). By gathering information about potential social partners, an individual can maximize the benefits and minimize the costs of social interaction and hence such information gathering will facilitate social decision-making and guide social interactions (O'Connell & Hoffmann 2011, 2012a). Therefore, understanding how animals assess a potential social partner provides a valuable complement to research on group joining or sociality. While understanding the extent of, and propensity towards sociality provides insight into social motivation, social partner assessment provides information about the behavioural mechanisms that animals use to determine social relationships once they have come into contact with one another.

Aggression within resource contests provides an excellent test bed for the study of social partner assessment. In a contest situation, the contesting individuals will typically be asymmetrical in some characteristics (e.g., their phenotype or motivational state), and hence the ability to assess the characteristics of an opponent is often thought to influence the contesting animals behaviour and decision-making processes (Arnott & Elwood 2009; Elwood & Arnott 2012). Opponent attributes may influence both the strategic (whether to engage in the first place, persist or withdraw from a fight) and tactical (what behaviours to employ) decisions within the fight (Briffa & Sneddon 2010).

Conflicts over resources are an inevitable consequence of the fact that resource supply is almost always outstripped by resource demand (Archer 1988). Whenever the conflict over resources between two conspecifics is resolved by direct interaction, the result is a resource contest (Huntingford & Turner 1987). Resource contests have been well studied and represent the first and one of the most successful applications of game theoretical models to the study of animal behaviour (Maynard-Smith & Price 1973; Maynard-Smith & Parker 1976; Maynard-Smith 1982; Reichert 2013). In general, we know that aggressive contests are inherently costly, taking up time and diverting energy away from alternate pursuits such as mating, foraging, or predator vigilance. In addition, aggression within a contest can sometimes result in injuries or even death (Enquist & Leimar 1990; Jacobbson et al. 1995; Brick 1998; Neat et al. 1998; Briffa & Elwood 2004). Because of the substantial costs of fighting, animals are expected to have adaptations to reduce the impact of these costs and streamline the process of conflict resolution (Parker 1974; Parker & Rubenstein 1981; Enquist & Leimar 1983; Hurd 1997; Maynard-Smith & Harper 2003; Arnott & Elwood 2009; Elwood & Arnott 2012).

One of the simplest ways to determine when to end a contest is to fight up until the point where some maximum cost threshold is reached, (Mesterton-Gibbons et al. 1996; Payne and Pagel 1996, 1997; Payne 1998). This process of ‘pure self-assessment’ has been identified in many species and may be a more widespread approach to fighting than has been previously appreciated (Taylor & Elwood 2003; Arnott & Elwood 2009). The simplicity of such a pure self-assessment contest strategy and the attendant low cognitive demands of fighting in this way are inherent advantages of this decision-making system. However, substantial pitfalls are also involved, because fighting based on self-assessment will lead losers to pay their maximum acceptable cost every time they fight, regardless of their initial likelihood of winning (Arnott & Elwood 2009). Instead, an animal can fight more efficiently by gathering information about the characteristics of its opponent and make use of this information when deciding whether to persist or withdraw (i.e., contest strategy) or which specific behaviours to employ during the fight (i.e., contest tactics; Briffa & Sneddon 2010). If an animal has the ability to collect information about its opponent's fighting ability, then this information can be used to quickly modify and even terminate contests, sparing individuals some of the costs of fighting especially if they are unlikely to win (Enquist & Leimar 1983).

The degree to which information is gathered about social partners in a contest situation, and how the use of such opponent information influences the decision-making processes remain a hotly debated question in the study of animal aggression (Reichert 2013). Do animals gather information about their opponents fighting ability? Do animals behave as though they possess information about their own fighting ability? And if so, how is the information about own and opponent fighting ability compared or combined to make contest decisions? These questions form the basis of an active and ongoing research area on the nature of animal contests and my thesis contributes to this research area.

I argue that by better understanding social partner assessment and social decision making within the contest context, we may uncover the important organizing principles of hierarchical animal societies (Earley & Dugatkin 2010) and the mechanisms of social information use in other contexts outside of aggression (O'Connell & Hofmann 2011).

3. Group dynamics and social networks

To date the vast majority of research on social behaviour has been conducted on individuals or dyads (Pinter-Wollman et al. 2013). This is because individual behaviour can be easily quantified and manipulated, while quantifying how dynamic interactions scale up to entire social groups, or manipulating a whole social group poses a much more serious logistic challenge (Krause et al. 2007; Croft et al. 2008). However, social behaviour often occurs at the level of groups larger than two individuals, and so it is likely that investigating social behaviour in more complex and natural social contexts will lead to important insights (Pinter-Wollman et al. 2013).

Recently, the use of social network theory, a graphical and statistical framework that characterizes the relationships between multiple interacting individuals, has begun to be applied more widely by animal behaviour researchers (Krause et al. 2007; Croft et al. 2008; Wilson et al. 2012, 2013; Pinter-Wollman et al. 2013). The social network approach allows researchers to visualize the relationship between individuals within groups through their interactions or via their spatial proximity to reveal the patterns of interactions or associations at the level of the social group (Flack et al. 2005, 2006; Wey et al. 2008). The social network approach offers a clear path for researchers to apply the lessons learned from studying social behaviour in dyads to a more complex and natural social situation (Wey et al. 2008; Pinter-Wollman et al. 2013).

Throughout this thesis I argue that aggression and, on the converse, submission, are key factors that dictate the structure of a group and define the hierarchical organization of animal societies (Dugatkin 1997; Bergmüller & Taborsky 2005; Earley & Dugatkin 2010). By studying the rules of animal contests, investigating aggressive and submissive interactions within groups, and exploring how these rules and interactions manifest at the level of the group, we can gain insight into the organizing principles of the social dynamics and hierarchy formation that typify most complex animal societies (Dugatkin 1997; Hsu et al. 2006; Cant et al. 2006; Buston & Cant 2006; Earley & Dugatkin 2010).

4. Oxytocic regulation of social behaviour

There are many neurophysiological mechanisms that appear to regulate behaviour in general, and social behaviour in particular (Becker et al. 2002). Over the past 20 years or so, the neuropeptide oxytocin and its non-mammalian homologues have received a great deal of attention as a potential modulator of social behaviour. The oxytocin system appears to be a key factor in the generation and regulation of social behaviour (Lee et al. 2009).

The oxytocin nonapeptide family is an evolutionarily ancient and highly conserved set of neuropeptide hormones (Insel & Young 2001; Goodson 2005, 2008, 2013; Donaldson & Young 2008; Lee et al. 2009; Ross & Young 2009). Oxytocin dates back to a duplication of the vasotocin gene in the ancestor of jawed fish and is found in some subtly varied form in all extant vertebrates (Hoyle 1999). Oxytocin is produced primarily in the hypothalamus within a set of highly conserved neuron populations, but is released throughout the brain via a complex network of projections and receptors that show remarkable variability and evolutionary labiality between species (Goodson & Thompson 2010). Oxytocin is also released into the periphery via the pituitary gland (Norris 2007). In the body, oxytocin acts as a hormone, which regulates smooth muscle contraction, cardiac function, parturition, and milk letdown (Norris 2007; Lee et al. 2009). In the brain, oxytocin acts as a broad-scope neuromodulator and plays a role in a diverse array of behaviours, including decision-making, reward, and the stress response (Ross & Young 2009; Churchland & Winkielman 2012). Of particular note, oxytocin seems to be germane to the regulation of specifically social behaviours including prosocial motivation, affiliation, pair-bonding, parental care, social memory, trust and love (Lee et al. 2009; MacDonald & MacDonald 2010). As a result, oxytocin is a key component of what has been termed the social decision-making network, which integrates the neural circuits involved in both the social brain network and the decision-making network and may be a key point of overlap between these interrelated neural systems (Goodson 2005; O'Connell & Hofmann 2011, 2012a).

As a result of its apparent role in the regulation of social behaviour across such an impressive diversity of social contexts and taxa, it is likely that evolution acting on the oxytocic hormone system is important for the emergence and divergence of social systems (Goodson 2008, 2013; Goodson & Thompson 2010; Goodson & Kingsbury 2011). By examining differences between individuals, populations and species in the expression of oxytocin, it is likely that we will gain insight as to the proximate control of divergent social lifestyles (Goodson 2013).

In teleost fish, the oxytocin homologue is known as isotocin. Isotocin differs from oxytocin at two of nine amino acid positions (Hoyle 1999; Godwin & Thompson 2012). Isotocin has received much less research attention than has oxytocin or mesotocin (the oxytocin homologue found in birds, amphibians and non-avian reptiles). Experimental manipulations of the isotocin system are particularly scarce (see Thompson & Walton 2004; O'Connell & Hofmann 2012; Braida et al. 2012 for a few rare examples). The existing evidence does suggest that isotocin serves very similar functions in regulating social behaviour in fish as oxytocin does in mammals and mesotocin in birds (Goodson et al. 2009; Godwin & Thompson 2012). Given that fish account for by far the most diverse vertebrate group (Nelson 2006), they are likely to offer important clues to the evolution of the oxytocin system and to the ancestral vertebrate condition (Butler & Hodos 2005). Therefore more research on the role of isotocin in regulating fish behaviour is clearly warranted (Godwin & Thompson 2012). The sheer diversity of social systems that exist among fish, and often among closely related species opens up the opportunity for comparative work examining the interplay between evolution acting on the isotocin system the emergence of divergent social systems.

5. Study species: Neolamprologus pulcher

Neolamprologus pulcher is a substrate-spawning cichlid, endemic to Lake Tanganyika, East Africa (Konings 1998) and is one of the most successful and abundant species in the lake. This fish species is distributed throughout the rocky littoral zone circumscribing the entire lake at a depth of 3-30 m (Konings 1998). *N. pulcher* are obligately social, living in permanent groups of up to 20 adults (Taborsky & Limberger 1981; Heg et al.2005). Each social group occupies a territory composed of a series of chambers and tunnels excavated out of sand beneath a pile of rocks or in the cracks of rocky ledges (Balshine et al. 2001, Fig. 1a). *N. pulcher* live in colonies, with many social groups in close proximity (Stiver et al.2007). These cichlids feed on zooplankton, which they forage for in the water column (Taborsky 1984). Their feeding areas extend from directly above their territories to a meter or so above the floor of the lake (Taborsky 1984, 1985, Werner et al.2003, Bruintjes et al.2010). There is little to no competition for food in the large feeding aggregations that form above the colonies (Wong & Balshine 2011a). By contrast, *N. pulcher* are fiercely territorial when they are near their rocky shelters on the substrate, and will aggressively guard their domain from both intruding conspecifics and other fishes (Taborsky 1984, 1985; Balshine et al. 2001; Desjardins et al. 2008).

N. pulcher groups are highly hierarchical; the largest male and largest female are dominant and monopolize the vast majority of reproduction (Wong & Balshine 2011a). Other group members are subordinate to the breeding pair and form a size-based dominance hierarchy (Wong & Balshine 2011b, Fig. 1b). Subordinate group members may act as helpers-at-the-nest, providing assistance to the breeding pair in brood care, territory maintenance and brood and territory defence (Taborsky & Limberger 1981; Taborsky 1984, 1985; Balshine-Earn et al. 1998; Wong & Balshine 2011a). As such, the mating-system of *N. pulcher* can be characterized as cooperatively breeding (Taborsky & Limberger 1981; Wong & Balshine 2011a). *N. pulcher* show a sex difference in life-history, males are typically the dispersing sex, switching groups prior to reaching breeding status while females are philopatric and often remain in their natal group throughout their lives (Stiver et al. 2007).

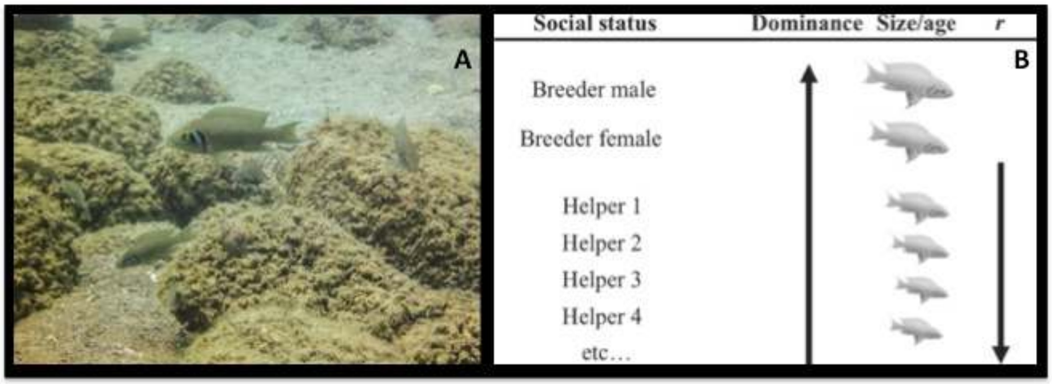

Fig. 1. A) A wild *Neolamprologus pulcher* group above their territory. B) A graphical depiction of the size based dominance hierarchy that characterizes a *N. pulcher* social group. Smaller fish are younger and more likely to be related (r) to the rest of the group than larger, older fish as a result of frequent breeder turnover and immigration.

N. pulcher are emerging as an exciting model system for the integrative study of social behaviour (for a recent review of *N. pulcher* research see Wong & Balshine 2011a). *N. pulcher*, unlike other cooperatively breeding vertebrates, are small bodied, short-lived and adapt very well to life in a laboratory environment. These characteristics make *N. pulcher* highly amenable to both controlled behavioural study in the laboratory and more naturalistic studies in the wild. Behavioural, physiological, developmental, neural and genomic tools are increasingly being adapted for and applied to *N. pulcher* (Buchner et al. 2004; Stiver et al. 2005, 2009; Aubin-Horth et al. 2007; Mileva et al. 2009; Taves et al. 2009; Arnold & Taborsky 2010; Taborsky et al. 2012a,b; O'Connor et al. 2013), suggesting that *N. pulcher* research will continue to grow and contribute in important ways to the integrative biology of social behaviour among other topics.

6. Aims of the thesis

In my thesis, I examine social behaviour and group structure in the highly social, cooperatively breeding cichlid fish, *Neolamprologus pulcher*. I take an integrative perspective and investigate both the ultimate factors favoring particular social behaviours and the proximate mechanisms that regulate those behaviours. Specifically, my thesis research focuses on two basic domains of social behaviour with an eye to social decision-making in each context: 1) grouping behaviour or sociality and 2) opponent assessment within resource contests. From a proximate perspective, I examine the role of the highly conserved nonapeptide hormone isotocin plays in regulating these behaviour and decision-making in these social contexts. In my first data chapter, Chapter 2, I examine grouping decisions in *N. pulcher* and sex differences in the degree of sociality. In Chapters 3 and 4, I examine opponent assessment and decision-making in general during *N. pulcher* resource contests. In chapters 5 and 6, I examine the role of isotocin in modulating sociality, decision making during resource contests, and behaviour within naturalistic social groups. In my final data chapter, Chapter 7, I use a social network approach to test hypotheses about the organizational principles of *N. pulcher* social groups. Finally, in Chapter 8, I provide a general discussion, which ties together ideas across the data chapters and examines more closely some of the issues and questions raised by my empirical work. Collectively, these papers contribute to our understanding of social behaviour and social decision-making using an emerging model system for the integrative study of social behaviour.

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Chapter 2: Sex differences in group-joining decisions in social fish

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Sex differences in group-joining decisions in social fish

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In social animals, decisions about which group to join can have important fitness consequences and may be particularly critical when groups have a strict dominance hierarchy that relates to reproductive success. Choosing a large group may maximize safety, but choosing to join a small group can minimize the delay until a dominant reproductive position can be reached. We explored this trade-off between safety and rank using *Neolamprologus pulcher*, a cichlid fish in which individuals conform to a rigid within-group dominance hierarchy. In this species, females typically inherit dominant positions by ascending in rank, while males commonly take over a dominant breeding position by dispersing into a new group. Because females have fewer opportunities to switch groups, we predicted that females would place higher value on social rank within their group than would males. To test this, we examined male and female *N. pulcher*'s preferences for joining large groups at a low rank versus joining small groups at a high rank. Males showed clear preferences for larger (presumably safer) groups, while females showed no such preferences. In a second experiment, we held joining rank constant, and found that both males and females showed a strong preference for large groups. Our results suggest that when joining a group, females consider both rank and safety whereas males are primarily concerned with safety. Our results help to elucidate the factors underlying social decision making in a cooperative breeder.

Group membership decisions are of critical importance to social animals (Krause & Ruxton 2002). Membership in a larger group may provide more effective and efficient vigilance (Elgar 1989; Roberts 1996; Uetz et al. 2002), dilute predation risk (Hamilton 1971; Wrona & Dixon 1991), augment mating opportunities (Westneat et al. 2000) and increase foraging success (Drent & Swierstra 1977; Brown 1986; Creel & Creel 1995). However, living in a larger group may also impose certain costs, including increased conspicuousness to predators (Lindström 1989; Cresswell 1994), more competition for food or mates (Janson & Goldsmith 1995) and increased transmission of disease (Hoogland 1979; Brown & Brown 1986). The trade-off between these costs and benefits poses a major life history decision for group-living animals (Krause & Ruxton 2002). Decisions about what size of group to join or whether to allow new members into the group are of particular importance in groups with pronounced dominance hierarchies (Krause & Ruxton 2002; Ang & Manica 2010; Jordan et al. 2010a, b), especially when the social hierarchy represents a queue to breed (Buston & Cant 2006; Wong et al. 2008; Wong 2010; Wong & Balshine 2011a). Larger groups may provide greater safety, but also present a longer and more difficult path to dominant breeder status (Kokko & Ekman 2002; Ang & Manica 2010). To date, most of the empirical studies examining group size preferences have been conducted in species without long-term dominance hierarchies (see Krause & Ruxton 2002 for a comprehensive review) and hence the potential trade-off between rank and safety has not received much attention.

Neolamprologus pulcher are a highly social, cooperatively breeding cichlid fish endemic to rocky littoral habitats in Lake Tanganyika, Africa (Taborsky & Limberger 1981; Taborsky 1984, 1985; Balshine-Earn et al. 1998; Balshine et al. 2001). *Neolamprologus pulcher* live and breed in stable social groups consisting of a single dominant breeding pair and between 1 and 20 subordinate helpers (Balshine et al. 2001; Heg et al. 2005). Subordinate group members assist the breeding pair by maintaining the territory (removing sand, debris and snails), defending the brood chamber and participating in direct care of the brood (Taborsky & Limberger 1981; Balshine et al. 2001). Individuals in *N. pulcher* groups form a strict linear size-based dominance hierarchy (Balshine-Earn et al. 1998; Fitzpatrick et al. 2008; Wong & Balshine 2011b). The largest male and largest female in each group form a breeding pair and all other group members are reproductively suppressed (Fitzpatrick et al. 2006). Social groups appear to serve a predominantly defensive function (Heg et al. 2004), as the predation pressure in *N. pulcher*'s natural habitat is formidable

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(Balshine et al. 2001; Heg et al. 2004; Wong & Balshine 2011a). As a result of *N. pulcher's* small size (<8 cm adult body length) and adaptability to laboratory conditions where they will perform their full suite of natural behaviour, they are amenable to controlled experimentation that is not ethical or feasible in other cooperatively breeding vertebrates (Wong & Balshine 2011a).

Subordinate *N. pulcher* have three possible routes to breeder status (Jordan et al. 2010a; Wong & Balshine 2011a): (1) they can remain in their current group as a subordinate helper in a queue to breed (Kokko & Johnstone 1999), (2) they can disperse and join another group in order to take over the dominant breeding position (Kokko & Ekman 2002) or (3) they can disperse and join another group as a subordinate helper thereby entering another breeding queue (Bergmüller et al. 2005). By leaving its current group and joining another group with a shorter queue (i.e. one with fewer larger same-sex individuals), a subordinate may expedite its ascent to breeding position (Kokko & Ekman 2002; Stiver et al. 2004). However, by joining a group with fewer members or fewer larger group members, a subordinate may sacrifice the inherent safety of a large group (Balshine et al. 2001; Heg et al. 2004). Jordan et al. (2010a) found that when faced with a choice between groups of the same size, *N. pulcher* subordinates prefer to join a group containing larger, more dominant fish, despite the fact that they were subject to higher levels of social aggression in these groups. These results suggest that *N. pulcher* may favour safety over high social rank. If *N. pulcher* always value safety over rank, then we would expect individuals to prefer to join a larger group in a lower rank position than to join a smaller group at a higher rank.

Differences in the route to dominant breeding status between males and female may generate sex differences in group-joining rules (Krause & Ruxton 2002). *Neolamprologus pulcher* represent a ‘mammalian type’ cooperative breeder (Russell & Lummaa 2009) in that females typically remain in a matrilineal queue and inherit breeder status in their natal group, whereas males often must disperse into a new group before attaining the breeder rank (Stiver et al. 2004, 2006). Males may be more attuned to group demographics (i.e. group composition and/or group size), as they are more likely to face a decision between joining different groups that vary in these parameters (Stiver et al. 2004, 2006, 2007). Alternatively, because females lack the option to disperse, they may place greater emphasis on their rank within the group, while males, because they can disperse, may place a higher value on safety. Female subordinates tend to be more active helpers within the group (Stiver et al. 2005; Desjardins et al. 2008a, b), which may suggest that females highly value their social position within the group.

In our first experiment, we examined the preference for small versus large groups of larger, same-sex conspecifics in subordinate adult *N. pulcher*. We assumed that larger groups would maximize safety while smaller groups would maximize the joiner's rank and would minimize the time until dominance ascension. We conducted preference trials for both males and females and explored sex differences in group size preference. Differences between males and females in their preference for large groups could mean that the sexes vary in the importance they place on safety versus rank or alternatively, in their sensitivity to cues of group size. To differentiate between these possible explanations, we conducted a second experiment in which we examined group size preferences when the joiner's rank was held constant regardless of which group it joined.

METHODS

Experimental Animals and Housing Conditions

All fish used in this experiment were laboratory-reared descendants of animals collected from Lake Tanganyika, Africa. Focal fish were 64 *N. pulcher* subordinate adults (standard length >4 cm, 32 males and 32 females) selected from 26 different social groups. Each social group from which these focal fish were taken consisted of a single dominant breeding pair and between 2 and 10 subordinate helpers of varying size (1–7 cm) housed in a 189-litre (92 × 41 × 50 cm) aquarium with two filters, two shelters and 3 cm of coral sand as substrate. Stimulus fish were selected from a large communal tank (183 × 48 × 60 cm; 527-litre), which contained approximately 80 adult *N. pulcher*. Water temperature in all aquaria was held constant at 25 ± 2 °C within chemical parameters that simulated the natural habitat of this species. All fish were fed ad libitum once a day, 6 days a week with dried or frozen prepared cichlid foods.

Testing Apparatus

The preference testing apparatus consisted of a large glass aquarium (90 × 44 × 38 cm; 150-litre), filled with 20 cm of conditioned water. Two smaller glass aquaria (40 × 20 25 cm; 20-litre), also filled with 20 cm of water, were placed inside and pushed against opposite ends of the larger aquarium. These smaller aquaria served as group stimulus chambers and ensured that no chemical cues were transferred between the focal fish and the stimulus fish. Opaque plastic barriers visually isolated the stimulus chambers from one another but allowed the focal fish to freely move between choice zones and inspect both stimulus groups. A 10 cm area (corresponding to approximately two body lengths of the average focal fish) in front of each of the stimulus chambers was clearly marked and formed the choice zone for each stimulus group.

Procedure

In each trial, a single stimulus fish was placed in one of the stimulus chambers and three stimulus fish were placed in the other chamber. The chamber that received the large or small stimulus group was randomly assigned by means of a coin flip. Research on other freshwater fish species has shown that fish possess the numerical abilities to make discriminations of this type and magnitude (Agrillo et al. 2007; Dadda et al. 2009). In the first experiment ($N = 40$, 20 males and 20 females), all of the stimulus fish were larger than the focal fish and the focal fish always joined a group at the bottom of the dominance hierarchy (either in rank 2 in the small group or in rank 4 in the large group). In the second experiment ($N = 24$, 12 males and 12 females), stimulus fish were chosen so that the focal fish would be the second-largest individual (rank 2) regardless of whether it chose to associate with the small or the large group. Each focal fish was used only once. The stimulus fish were drawn with replacement from the same population for both experiments. Stimulus fish were changed after each trial, ensuring that focal fish were exposed to different combinations of stimuli. Only same-sex animals were used as stimuli in both experiments to ensure that grouping decisions represented a form of social partner choice (sensu Dugatkin & Sih 1995) and not mate choice. The focal fish were always unfamiliar with the stimulus fish. Outside of those criteria, stimulus fish were selected at random from the fish in the communal tank.

Once the stimulus fish were in place, a focal fish was removed from its social group and introduced into the central choice chamber of the preference apparatus. We allowed the focal fish and the stimulus fish to acclimate to the apparatus for 5 min. During this acclamation period, the focal fish could freely swim about the apparatus and view both stimulus groups. Following the acclimation period, we filmed the apparatus for 10–15 min (15 min in experiment 1; 10 min in experiment 2). After the first observation, we stopped filming for an interobservation interval (40 min in experiment 1; 30 min in experiment 2) during which the focal fish remained undisturbed and could continue to view the stimulus fish. After the interobservation interval, we resumed filming for an additional 10 min (second observation period). Previous research on this species has shown that short-term preferences in affiliating with one group over the other predict the ultimate decision to join that group (Jordan et al. 2010a). After the second observation period, the focal and stimulus fish were returned to their respective home tanks. All trials took place between 1100 and 1600 hours. One female managed to escape from the central group-preference area during the first observation period in the first experiment, so the data for that observation was excluded.

Behavioural and Statistical Analyses

A trained observer, blind to the sex of the fish and study hypotheses, scored each trial from the video recordings. We divided each observation period into 5 min blocks to explore the consistency of behaviour throughout each trial. During each of the 5 min blocks, we recorded the time (in seconds) that each focal fish spent with the majority of its body inside each of the two choice zones (within approximately two body lengths of each stimulus chamber). We also scored the number of times that each focal fish switched from one choice zone to the other.

During both experiments, we recorded the activity of each stimuli group (scored as the number of movements of greater than one body length made by each stimulus fish), because stimulus activity levels can affect group preferences (Gómez-Laplaza 2006). We also recorded the number of aggressive acts the stimulus fish directed towards the focal choosing fish in experiment 2.

All group preference and stimulus fish activity data were normally distributed and were analysed with two-tailed parametric statistics. We compared the proportions of males and females that preferred the large group using a chi-square test. To compare the time that each focal fish spent in proximity to the large group versus the small group and any sex differences in this relationship, we performed an ANOVA with time spent in each of the two choice zones during each 5 min observation block as the within-subjects factor and sex as a between-subjects factor. The social group from which the focal fish originated had no effect on the time spent in either choice zone (one-way ANOVA with social group as a random effect); hence, subjects were combined across social groups for all analyses. We compared the activity of the small and large stimulus groups using an ANOVA, with group size as a within-subjects factor and sex as a between-subjects factor. Data on aggressive behaviour directed by the stimulus fish towards the focal fish in experiment 2 were not normally distributed and were analysed using a Wilcoxon signed-ranks test.

Ethical Note

No animals sustained any injuries or showed signs of undue stress during or after the experimental procedure. Methods described for animal housing and handling were assessed and approved by the Animal Research Ethics Board of McMaster University (Animal Utilization Protocol No. 10-11-71) and adhered to the guidelines of the Canadian Council for Animal Care.

RESULTS

Experiment 1: Join a Large Group at Low Rank or Join a Small Group at High Rank?

In total, 17 of 20 males and 12 of 20 females spent more time with the large group. The proportion of males that preferred the large group tended to be greater than the proportion of females that did so, but this difference was not significant (chi-square test: $\chi^2_1 = 3.14$, $P = 0.08$). Across all fish (males and females combined) there was a strong preference for the large group (ANOVA: $F_{1,36} = 10.88$, $P = 0.002$), suggesting that *N. pulcher* strategically choose larger groups even though it would mean assuming a lower rank. There was no effect of observation time point ($F_{4,144} = 0.47$, $P = 0.76$), indicating that the fish's preference for affiliating with the large stimulus group over the small one was stable across all the observation blocks. There was no effect of sex ($F_{1,36} = 2.114$, $P = 0.16$) in this analysis, indicating that males and females spent similar amounts of time on average in association with conspecifics. Interestingly, the interaction between sex and group size preference verged on significance ($F_{1,36} = 3.77$, $P = 0.06$), suggesting that male and female *N. pulcher* may allocate their time differently between the two kinds of groups.

The near-significant interaction between sex and group preference along with our a priori expectations about sex differences led us to analyse the preferences for groups separately for each sex. When analysed separately, males strongly preferred to affiliate with the large group over the small group (ANOVA: $F_{1,18} = 12.416$, $P = 0.002$; Fig. 1a), while females spent similar amounts of time with the large and small groups ($F_{1,18} = 1.03$, $P = 0.32$; Fig. 1b), indicating that the overall strong group preference reported above was driven by males.

Males and females made a similar number of switches between choice zones (mean ± SE: males = 14 ± 2; females = 13 ± 2; Welch's *t* test: $t_{36.8} = 0.55$, $P = 0.59$). Overall there was more total activity in the large stimulus group (measured as the mean ± SE per-minute rate of movement: large group = 8.7 ± 0.4; small group = 3.6 ± 0.3; ANOVA: $F_{1,38} = 131.39$, $P < 0.001$), but the per-individual activity was significantly higher in the small stimulus group (mean ± SE: large group = 2.9 ± 0.1; small group = 3.6 ± 0.3; $F_{1,38} = 8.63$, $P = 0.006$). There were no sex differences observed in

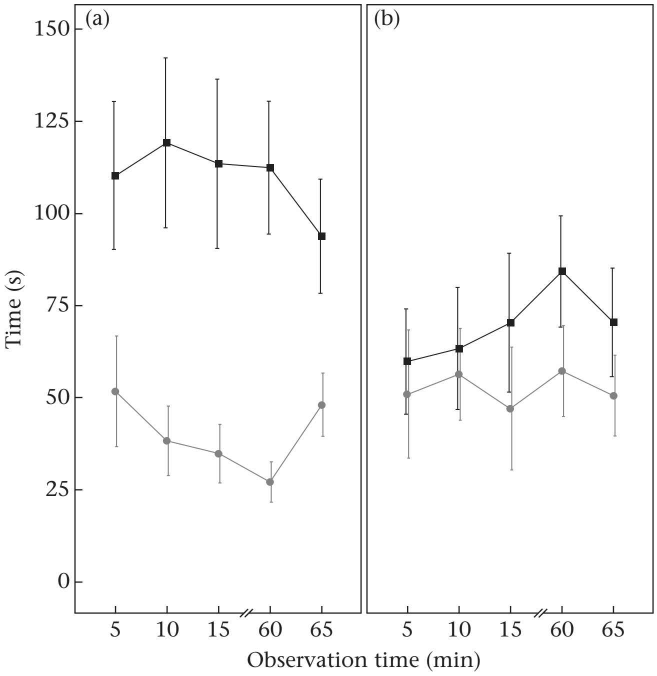

Figure 1. Experiment 1. Mean ± SE time spent (in seconds) in association with the small (circles and grey lines) and large (squares and black lines) stimulus groups in 5 min blocks during the first (0–15 min) and second (60–70 min) observation periods for (a) males and (b) females.

either measure (total activity: $F_{1,38} = 0.05$, $P = 0.83$; activity per fish: $F_{1,38} = 1.29$, $P = 0.26$).

Experiment 2: Join a Small or a Large Group When Rank is Held Constant?

In total, 11 of 12 males and 9 of 12 females preferred the large group (the proportion of males versus females that preferred the large group was similar, Fisher's exact test: $P = 0.59$). Across all individuals, there was a strong preference for the large group (ANOVA: $F_{1,22} = 19.14$, $P < 0.001$; Fig. 2) and this preference was stable over time ($F_{3,66} = 1.54$, $P = 0.21$). The effect of sex was not significant, indicating that males and females spent similar amounts of time associating with the social groups ($F_{1,22} = 0.61$, $P = 0.44$). The sex by group-preference interaction was not significant, indicating that males and females did not differ in their preference for the large group ($F_{1,22} = 1.18$, $P = 0.29$). Their strong preference for large groups was maintained when each sex was examined separately (males: $F_{1,11} = 14.62$; $P = 0.003$, Fig. 2a; females: $F_{1,11} = 5.52$, $P = 0.04$, Fig. 2b).

Males and females did not differ in the number of switches between the choice zones (mean ± SE: males = 8.3 ± 1.8; females = 10.0 ± 1.3; Welch's *t* test: $t_{20.5} = -0.76$, $P = 0.45$). As in the first experiment, the large stimulus group was more active overall (ANOVA: $F_{1,22} = 20.62$, $P < 0.001$) and the per-individual activity was greater in the small group ($F_{1,22} = 4.52$, $P = 0.045$). Among the stimulus fish, there were no sex differences in activity (total activity: $F_{1,22} = 0.04$, $P = 0.85$; activity per individual fish: $F_{1,22} = 0.743$, $P = 0.40$). There was no difference between the small and large groups in the number of aggressive acts directed towards the focal fish (Wilcoxon signed-ranks test: $Z = 0.00$, $N = 24$, $P = 1.00$) and the overall rates of aggressive behaviour were extremely low (mean ± SE = 0.05 ± 0.01 aggressive acts/min).

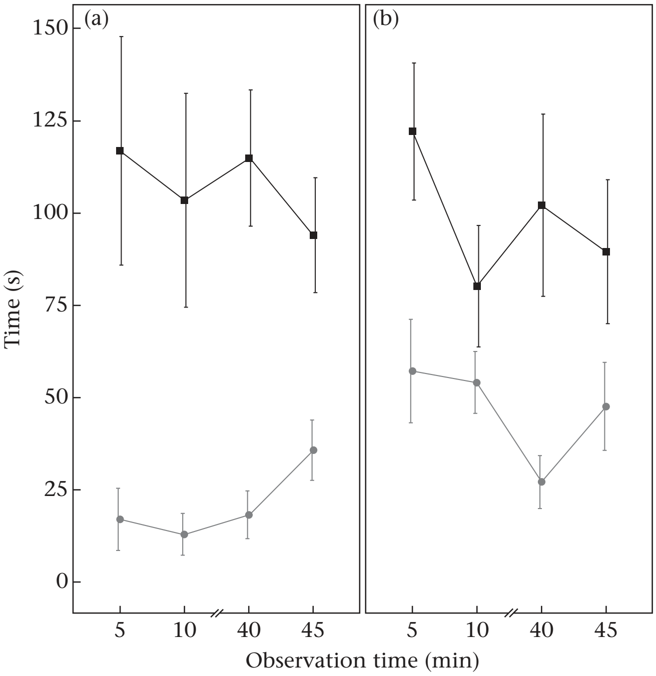

Figure 2. Experiment 2. Mean ± SE time spent in association with the small (circles and grey lines) and large (squares and black lines) stimulus groups in 5 min blocks during the first (0–10 min) and second (40–50 min) observation periods for (a) males and (b) females.

DISCUSSION

Male *N. pulcher* showed a strong and consistent preference for associating with a large group regardless of the rank they would assume when joining that group. Females by contrast, preferred large groups only when they could join that group at a high rank. The sex differences in the preference for large groups in the first experiment did not stem from a general difference in sociability between the sexes as males and females spent a similar amount of time socializing during both experiments. Given the highly social nature of *N. pulcher*, it is not surprising that both sexes preferred to be near conspecifics.

Deciding which group to join is critically important in *N. pulcher* as the social group is organized into a size-based linear dominance hierarchy with the most dominant pair monopolizing reproductive output (Wong & Balshine 2011a). In our first experiment, all of the stimulus fish were larger than the focal fish. Therefore, the focal fish would be in a relatively better social position by affiliating with the single stimulus fish (rank 2) rather than the group of three stimulus fish (rank 4). On the other hand, there is good evidence to suggest that one of the primary benefits of group living in *N. pulcher* is the abatement of predation (Balshine et al. 2001; Heg et al. 2004). By affiliating with a larger group, the focal fish may be choosing greater safety over improved social standing. In a previous study, Jordan et al. (2010a) found that *N. pulcher* preferred to associate with groups that had larger helpers over groups with smaller helpers. By choosing a group with small helpers, the focal fish could have improved its social standing, but groups with large helpers were preferred presumably because these groups provided greater safety from predators. In larger groups, individuals have lower per-individual risk of predation than they do in small groups (Balshine et al. 2001), possibly due to shared vigilance, selfish herd effects, predation risk dilution and/or predator confusion effects (Krause & Ruxton 2002). Alternatively or additionally, larger groups may be better buffered against predation because mutualistic territory defence is more effective in these groups (Krause & Ruxton 2002; Wong & Balshine 2011a). *Neolamprologus pulcher* helpers stage a more vigorous defence against predators than they do against unfamiliar intruding conspecifics (possible joiners and rank competitors) and prioritize defence against predators over defence against conspecific intruders (Desjardins et al. 2008a). Taken together with our results, these findings suggest that *N. pulcher* subordinates, particularly males, may prioritize safety over rank.

Preference for larger groups may also relate directly to the evolution of cooperative breeding in *N. pulcher*. The group augmentation hypothesis predicts that helpers provide help in cooperative breeding systems in order to produce helpers that will assist their own breeding efforts when they eventually assume the breeding position (Woolfenden 1975; Woolfenden & Fitzpatrick 1978; Kokko et al. 2001). If group augmentation is an important force in the evolution of helping behaviour in this species, then *N. pulcher* individuals may prefer to join large groups rather that small ones because large groups are more likely to be large when these joining fish eventually ascend to the breeding position (Kokko et al. 2001). Larger *N. pulcher* groups are more productive than smaller ones, and breeders with more helpers have reduced workloads and increased feeding rates compared to those with fewer helpers (Balshine et al. 2001). Heg et al. (2005) found that territories with large groups in one year continued to contain large groups in the next year, suggesting that large groups are less likely to go extinct (Heg et al. 2005). Future research with marked individuals and territory quality controls would provide further confirmation of this result.

The sex difference we observed in our first experiment could be a result of male-biased dispersal in *N. pulcher* (Stiver et al. 2004, 2006, 2007). Males may be more attuned to assessing group composition because they often leave home and choose a strange group with which to affiliate, so joining is a normal part of male life history and developmental trajectory. In contrast, females rarely join unfamiliar groups, and remain in or near their natal territory; hence, females may not as readily make use of group size cues. However, our second experiment suggests that females do in fact pay attention to cues of group size but are also concerned with their rank when joining a group. When joining rank and group size were in conflict, females showed no clear preference, indicating they may consider both parameters (Wong & Rosenthal 2005). When joining rank was held constant though, females preferred the large group. Female philopatry may have selected females to be more sensitive to their rank position within a group as they lack any alternative route to breeding position. Males by contrast are able to disperse and breed in another group if their current situation is unfavourable upon reaching breeding size.

Male and female *N. pulcher* are equally susceptible to predation (Balshine et al. 2001) and experience similar levels of within-hierarchy conflict (Wong & Balshine 2011b), so differences in predation risk or costs of being subordinate are unlikely to explain the sex difference we observed. Females, being philopatric, may benefit more from group augmentation than do males because they spend a larger portion of their life in the group in which they eventually breed (Kokko et al. 2001). As a result we might expect females to have a stronger preference for large groups; however, we found the opposite, suggesting that sex differences in the benefits of group augmentation do not account for our results. Similarly, there is some evidence that high-ranking male subordinates may be able to secure some reproduction within the group, at least in laboratory settings (Heg et al. 2006). Subordinate reproduction may increase the value of being a high-ranking male subordinate; however, we found that females pay more attention to social rank than males did. Future experimentation is required to conclusively rule out these alternative explanations.

When the importance of dominance rank has been investigated, dominance rank has been shown to affect grouping decisions. For example, Gómez-Laplaza (2005) found that juvenile angelfish, *Pterophyllum scalare*, preferred to associate with socially subordinate individuals from their own shoal. Wong & Rosenthal (2005) found that female swordtails (*Xiphophorus birchmanni* × *Xiphophorus malinche*) preferred to associate with large groups over small groups and with similarly sized individuals over differently sized individuals, but when preferred group size and preferred body size were placed in conflict, swordtails showed no clear choice, suggesting they valued both parameters equally. This result mirrors our findings in females, where conflicting preferences between group size and composition can lead to indecisiveness or individual variation in group-joining preferences. In contrast, Krause & Godin (1994) found that banded killifish, *Fundulus diaphanous*, prefer large to small groups and like-sized individuals to differently sized individuals, but these fish prioritize body size over group size when making grouping decisions. The results of the banded killifish study illustrate that group joiners may differentially value the characteristics of a social group. This may be the case with *N. pulcher* males that appear to place a higher value on group size than on joining rank. Neither swordtails nor banded killifish have rigid dominance hierarchies, and these results together with the current study show that group choice decisions may be handled differently between sexes and across species with different social systems, suggesting that social context plays an important role in group size preferences. The neural basis of group-joining preferences (e.g. neuropeptides in the oxytocin family; Goodson et al. 2009) appears to be highly conserved between taxa (Goodson 2005), and different social systems may evolve through the modulation of these systems (Goodson 2008).

In conclusion, males and females did not differ in their general preference to be near conspecifics, suggesting that the sex differences in grouping decisions are not due to sex differences in desire to be near conspecifics. Males appear to base group-joining decisions on concerns for safety rather than social position, whereas females seem to consider both rank and safety. Females may be more sensitive to concerns over their rank because they typically inherit a dominance position and have less opportunity to pursue alternative options. Future research should examine how cues of predation risk affect group-joining decisions. If grouping is primarily an antipredator response, then predation risk cues should increase preference for large groups. Ultimately, the structure of the group is modulated by the preferences of both joining and current members (Jordan et al. 2010b). Investigating how individuals within a group make the decision to admit new members would nicely complement our current study and would be a productive avenue for future research.

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Chapter 3: Rules of engagement for resource contests in a social fish

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Rules of engagement for resource contests in a social fish

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A rich theoretical framework exists for understanding animal conflict. When two opponents fight over a resource, the duration, intensity and outcome of the fight ought to be determined in large part by the relative difference in resource-holding power between contestants. While our understanding of one-time conflict resolution is excellent, our knowledge is still limited of how these rules scale up when contests occur in a social context where individuals have long-term interactions. Here, we use a convenient model system, *Neolamprologus pulcher,* a small cooperatively breeding cichlid fish, to explore decisions in pairwise contests over resources in a species where two individual contestants are likely to remain in the same social group, and regularly and repeatedly interact. Contests began after approximately 1 min, with a short display phase, and continued in an aphasic manner for an average of 10 min before a clear winner emerged. Information about opponents' body size was important when deciding on the giving-up point, but contestants' own body size was not, suggesting that assessment of opponent size is paramount in contest decision making. No sex differences were detected in contest structure, duration or intensity, and contests between males or between females were indistinguishable. These results offer an important window on conflict in a cooperative breeder and shed insight on rules of engagement within hierarchical social groups.

Contests occur whenever competition between two or more individuals is settled by direct interaction (Briffa & Sneddon 2010). Individuals fight to secure mating opportunities or any other limited resource and such resource contests are widespread throughout the animal kingdom (Parker 1974; Enquist & Leimar 1987; Huntingford & Turner 1987; Archer 1988; Arnott & Elwood 2008; Briffa & Sneddon 2010). Although group-living animals with pronounced dominance hierarchies are thought to have overall lowered aggression, contests may still be extremely important in these species because aggressive interactions establish an animal's position in the hierarchy and high dominance rank often leads to high fitness (Clutton-Brock et al. 1979; Jennings et al. 2004, 2005; Cant et al. 2006; Briffa & Sneddon 2010). Cooperative breeders are species that have a social system in which individuals help rear the offspring of other more dominant individuals and hence forgo or diminish their own reproductive efforts. Contest behaviour may be critical within cooperative breeders, where dominance rank is often closely linked to breeding opportunities (Earley & Dugatkin 2010). It is neither practical nor ethical to study contest behaviour in most cooperatively breeding vertebrates because of their typically large body size and/or prohibitive space required to house entire social groups. As a consequence, contest behaviour is rarely studied in these species (Elwood & Parmigiani 1992; Briffa & Sneddon 2010). In the current study, we aim to explore decision making during resource contests in a cooperatively breeding vertebrate using the convenient Tanganyikan cichlid, *Neolamprologus pulcher* (Taborsky & Limberger 1981).

Neolamprologus pulcher live in social groups consisting of a single breeding pair and on average five to seven subordinate helpers at the nest that form a size-based linear dominance hierarchy (Balshine-Earn et al. 1998; Balshine et al. 2001; Heg et al. 2005; Fitzpatrick et al. 2008). Groups live and breed in the rocky littoral zone and use excavated caves underneath rocks both as shelter from predators and as a nest for eggs and larvae (Taborsky 1984, 1985; Balshine-Earn et al. 1998). Predation pressure in *N. pulcher*'s natural environment is severe (Balshine et al. 2001; Heg et al. 2004), and access to a well-protected shelter is essential for reproduction and survival (Balshine et al. 2001; Heg et al. 2004). There is considerable competition for suitable shelters (Taborsky 1984; Bergmüller et al. 2005), and limitations on shelter availability may be a causal factor in the evolution of group living and cooperative breeding (Emlen 1982; Hatchwell & Komdeur 2000; Kokko & Ekman

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2002; Wong 2010). *Neolamprologus pulcher* frequently aggress against conspecifics in their social groups (Taborsky 1984; Desjardins et al. 2005; Taves et al. 2009; Balshine-Earn et al. 1998; Wong & Balshine 2010a); furthermore, predation or dispersal events regularly result in vacancies in the dominance hierarchy, allowing subordinates to advance in rank or assume a higher rank in another group, and during these events, escalated contests can erupt (Balshine-Earn et al. 1998; Stiver et al. 2006; Fitzpatrick et al. 2008). Because *N. pulcher* are small (<8 cm in adult body length) and adapt well to laboratory conditions, readily performing their full suite of natural behaviours in aquaria (Wong & Balshine 2010b), they offer a unique opportunity to stage controlled dyadic contests in a cooperatively breeding vertebrate (Riebli et al. 2011). Pairwise contests may offer an important window into social conflict resolution within social groups, and understanding the rules of engagement may shed light upon what information is important when making decisions within a social group (Cant et al. 2006; Cant & Johnstone 2009; Field & Cant 2009; Cant 2011; Wong & Balshine 2010a, b).

The simplest way to decide whether to persist in a contest and how hard to fight is to base these decisions on one's own capabilities (fighting ability: termed resource-holding power or potential, hereafter RHP; Parker 1974; Maynard Smith 1982). Such contests have been modelled and are known as 'pure self-assessment' models, where each individual has an RHP-dependent threshold cost that it can bear and will persist in the contest until its own cost threshold is reached (Mesterton-Gibbons et al. 1996; Payne & Pagel 1996, 1997). Individuals with higher RHP have higher cost thresholds and can persist longer and win resources (Briffa & Sneddon 2010). A modified form of self-assessment that allows for higher RHP individuals to also inflict higher costs upon their opponents is known as the cumulative assessment model (Payne 1998). Contests in a wide variety of taxa appear to be consistent with the logic of self-assessment (Bridge et al. 2000; Morrell et al. 2005; Prenter et al. 2006; Stuart-Fox 2006; Brandt & Swallow 2009).

The price of fighting according to a self-assessment rule is that losers will always pay their maximum threshold cost, even when fighting with a distinctly superior opponent. If a fight is clearly unwinnable, then it is best abandoned early to avoid the costs of fighting (Maynard Smith & Harper 2003), and natural selection is likely to favour individuals that gather information about their opponents and then apply this information during the contest (Enquist & Leimar 1983; Enquist et al. 1990). Hence, in mutual-assessment models, it is assumed that contestants compare the RHP of their opponent with their own RHP and act on this information (Enquist & Leimar 1983; Enquist et al. 1990; for some recent and excellent reviews of these models, see: Arnott & Elwood 2009a; Briffa & Sneddon 2010). Mutual assessment is both intuitively satisfying and the dominant paradigm used to explain animal contests (Taylor & Elwood 2003). A negative relation between RHP asymmetry and contest duration (although similar arguments apply to contest intensity or other measures of cost) has been used as the gold standard to support the notion that contests are settled by mutual assessment. When contestants are closely matched, the asymmetry in RHP is more difficult to detect, and a longer fight is required to determine the winner. Recently, Taylor & Elwood (2003) have shown that this relationship can be generated by the fact that loser RHP is necessarily low whenever asymmetry is substantial. They recommend examining the independent effects of winner and loser RHP on fight cost. Mutual assessment predicts that increasing winner and loser RHP will have equal and opposite effects on contest cost, with increasing loser RHP increasing the cost of a contest and increasing winner RHP decreasing it. Pure self-assessment predicts only the positive effect of loser RHP and no effect of winner RHP. In general, animals living in long-lasting social groups have ample opportunities to gather information about other group members, and mutual assessment mechanisms may be especially common in these systems (Briffa & Sneddon 2010).

Contest behaviour may differ between the sexes, as the rewards for (and costs of) conflict may vary between males and females (Trivers 1972). To date, the vast majority of research on aggressive interactions has focused on understanding male–male contests (Archer 1988). This sex bias is unsurprising, given that theory and empirical research show that males more commonly engage in conspicuous dangerous contests (Trivers 1972; Archer 1988). However, in many species, females too engage in contests (Ayer & Whitsett 1980; Archer 1988; Gowaty & Wagner 1988; Berglund et al. 1993), and when fight tactics have been investigated in both sexes, interesting differences are often revealed (Holder et al. 1991; Draud et al. 2004; Arnott & Elwood 2009b; but see Barlow et al. 1986; Koops & Grant 1993). Both male and female *N. pulcher* engage in resource contests (Desjardins et al. 2005; Taves et al. 2009), and there are reasons to predict they will behave similarly in contests. Male and female *N. pulcher* are rather monomorphic, and females are often described as being equally aggressive as males and generally masculinized (Aubin-Horth et al. 2007; Desjardins et al. 2008a, b; Wong & Balshine 2010b). However, males typically disperse prior to reaching dominant status, whereas females are more philopatric, often inheriting breeding status in their natal groups (Stiver et al. 2004, 2006, 2008). These different life history trajectories mean that the value of winning a shelter or dominance status may vary between males and females and that the selection pressure for aggressive behaviour in males and females may differ.

In the current study, we describe the structure of dyadic *N. pulcher* resource contests for both males and females, focusing on the information that each individual uses to make decisions. Collectively, we sought to understand the underlying logic of resource contests in a cooperative breeder, and in particular, to determine whether decision making based on self-assessment or mutual assessment provides the best fit with *N. pulcher* contest behaviour. Our ultimate goal was to improve our understanding of conflict resolution within small-scale animal societies, by elucidating the decision-making mechanisms used by a highly social animal in a conflict situation.

METHODS

Study Animals and Housing Conditions

We used 90 sexually mature *N. pulcher* (50 males and 40 females) in this study. The average standard length (SL; measured from the tip of the snout to the caudal peduncle) of the animals was 5.44 ± 0.09 cm (range 4.27–7.15 cm). All study animals were laboratory-reared descendents of wild-caught breeding stock collected from Lake Tanganyika. All fish included in this study were subordinate helpers taken from permanent social groups maintained in the laboratory. Each social group in the laboratory consisted of a single dominant breeding pair and 2–10 subordinate helpers of varying size (1–8 cm) and was housed in a 189 litre (92 × 41 × 50 cm) aquarium with 3 cm of coral sand for substrate, two terracotta flowerpot halves as breeding shelters and two large sponge filters. Water was held at a constant temperature of 25 ± 2 °C and kept within chemical parameters that mimic the natural environment of the species. The fish were exposed to a 14:10 h light:dark cycle prior to and during the study. Fish were fed daily ad libitum with prepared cichlid flakes prior to and during the course of the study.

In total, we staged 56 same-sex contests (26 female, 30 male). The study was conducted in two testing periods, the first of which ran from October 2008 to February 2009 and the second from April to August 2010. During the first testing period, 36 fish (18 males, 18 females) were used to stage 29 contests (15 female, 14 male). Some of the fish in the first test period were used in two contests, resulting in 12 contests that involved at least one fish that had previously fought. However, fish never fought the same opponent twice, were chosen randomly with respect to their winner or loser status in the previous contest and were given at least 2 weeks to recuperate within their social group between the two contests. So including the 1-week acclimation period in the testing apparatus, fish had a minimum of 3 weeks in between fights. The fish's extensive daily interactions with its social group between contests probably minimized any winner or loser effects from the previous contest (Hsu et al. 2006). In the second testing period, we used 54 fish (32 males, 22 females) to stage 27 contests (16 male, 11 female). Fish in the second testing period were used only once, and no fish from the first testing period was used in the second. All trials in both test periods were conducted between 0900 and 1200 h. We used SL as a proxy for RHP. Body size has been shown to be a good predictor of fight outcome and to accurately reflect RHP in cichlid fishes (Enquist et al. 1987, 1990; Koops & Grant 1993). Body mass of the fish was highly correlated with SL (Pearson correlation: $r_{89} = 0.92$), so we used SL in all analyses reported here. We computed the percentage size asymmetry for each contest using the following formula: size asymmetry $= |100((\text{loser SL}/\text{winner SL}) \times 100)|$.

Apparatus and Experimental Procedure

Contests were staged in 38 litre (50 × 25 × 30 cm) aquaria subdivided along their length into three equal compartments (each 16.5 × 25 × 30 cm) by two opaque plastic partitions. An opaque PVC pipe (6.50 cm long, 7.50 cm in diameter) was placed into each of two outer compartments as a shelter for the fish. A terracotta flowerpot half was placed in the central compartment. Each contest aquarium was supplied with 1.5 cm of coral sand as substrate. The light schedule and the water for the contest aquaria were maintained in the same conditions as in the social housing aquaria described above.

For each contest, two fish of the same sex (determined by examination of the external genitalia) and generally of similar size were selected haphazardly from two different social groups and placed into each of the two outer compartments of the contest aquarium. The fish in a contest were always unfamiliar with one another. Fish were marked for identification by clipping the dorsal fin in one or two of several possible positions. We routinely use this marking technique in our laboratory to identify individual fish. The marks are temporary, do not affect fish behaviour and are easily recognizable on videotape. The fish show no ill effects from the marking procedure and recover immediately.

Test fish were removed from their social group and placed in one of the two end compartments, visually isolated from their opponent for an acclimation period of 7 days. During this period, test fish could establish territorial ownership over his or her compartment and shelter. Following the week-long acclimation period, the shelter from each fish's end compartment was removed and then we simultaneously raised the two opaque plastic barriers, allowing the fish access to the shelter in the centre chamber as well as access to each other. *Neolamprologus pulcher* are highly territorial and will readily fight for access to a shelter (Desjardins et al. 2005; Taves et al. 2009). In most cases, a vigorous contest quickly began. Trials were stopped after 20 min. All trials were videotaped and later scored by an observer blind to the sex and body size measurements of the fish.

Scoring

When one fish fled from the other three consecutive times without retaliation, or consistently held a submissive posture (head held upwards and tail pointing downwards with unpaired fins folded tightly against the body) while avoiding the other fish, we declared that fish to be the loser and the other fish as the winner. If no fish had clearly emerged victorious within 20 min, we categorized the fight as a tie. For each contest, we scored onset time (the time it took for the fish to begin fighting after the barriers had been raised) and the duration of the display-only phase (the time from the first display to the first instance of physical contact). We also recorded the total duration of the contest, measured from the time the barriers were raised to the point at which the loser relented. The intensity of the contest was measured as the per-minute rate of aggressive behaviour for both fish combined.

Aggressive behaviour in *N. pulcher* is similar to what has been observed in other cichlids (Baerends & Baerends-van Roon 1950; Turner & Huntingford 1986; Barlow et al. 1986; Enquist et al. 1987, 1990; Koops & Grant 1993; Hurd 1997; Neat et al. 1998; Reddon & Hurd 2009). Following previous research in *N. pulcher* (Taborsky 1984; Hamilton et al. 2005; Bergmüller & Taborsky 2007; Mitchell et al. 2009; Riebli et al. 2011), we combined lateral displays (where the fish presents its lateral aspect to its opponent while extending its unpaired fins), frontal puffed throat displays (where the fish faces its opponent head-on and flares its opercula out to the side) and aggressive head-down postures (where the focal fish faces its opponent and lowers its head below the height of its tail, usually while extending its unpaired fins) into one general category of 'aggressive displays'. Likewise, we combined both rams (where the focal fish impacts its opponent with its head without biting) and bites into a single category of 'physical contact behaviour' (for detailed descriptions of the behavioural repertoire of *N. pulcher,* see Sopinka et al. 2009).

Statistical Analysis

All data were tested for normality and transformed when necessary (log + 1). Data were analysed using nonparametric equivalents when they failed to meet parametric assumptions. All 2 × 2 chi-square tests were Yates corrected (Yates 1934). We compared winner and loser size and behaviour using paired *t* tests and the content of male and female contests using Welch's *t* tests (as recommended by Ruxton 2006). We performed ANCOVA analyses to look at the relation between contest duration and intensity, with winner and loser size separately, and to look for correlations between winner and loser behaviour. We included sex in each of these models. Interactions were examined and dropped from the models if they were not significant. All values are presented as mean ± SE.

Ethical Note

Trials were carefully monitored by an observer situated 1.5 m away from the contest tank. Any contest in which fish sustained visible injury or appeared to be excessively distressed was stopped immediately. This criterion resulted in the premature stoppage of one male contest, reducing the total number of contests from 56 to 55. To minimize stress and the risk of injury to the fish (following the recommendation of Huntingford 1984) we limited the contests to a short duration and terminated all contests after 20 min. Following each contest, we inspected each fish for injury, and no animal sustained any visible damage. The methods described for animal housing, capture and marking were assessed and approved by the Animal Research Ethics Board of McMaster University (Animal Utilization Protocol No. 10-11-71) and adhered to the guidelines of the Canadian Council for Animal Care.

RESULTS

Contest Outcome

Forty-five of the 55 contests had a clear winner and loser. The remaining 10 trials (five of each sex) were undecided within the 20 min contest framework and were scored as ties. All further analysis focused only on the decided contests ($N = 45$).

Winners were larger than losers (paired t test: $t_{44} = -4.33$, $P < 0.001$). Larger fish won 32 of the 45 decided fights ($\chi_1^2 = 8.02$, $P = 0.006$). The tendency for the larger fish to win did not differ between males and females (Yates chi – square test : $\chi_1^2 = 0.08$, $P = 0.78$). In those contests where the contestants differed in body size by 5% or more, the smaller fish emerged victorious in only two of 24 instances; however, when the size difference was less than 5%, small fish won about half of the time (11/21 contests; Yates chi-square test: $\chi_1^2 = 8.54$, $P = 0.003$; Fig. 1).

Contest Structure

Contestants took an average of 51.56 ± 7.02 s to commence displaying (range 3–175 s), and there was no difference between the sexes in display onset time (Welch's t test: $t_{35.5} = 1.04$, $P = 0.31$). Contests frequently began with a near simultaneous display from both competitors, so it was often difficult to discern which fish initiated the contest.

Rates of aggression between winners and losers were significantly positively related (ANCOVA: $R^2 = 0.39$, $F_{1,42} = 25.43$, $P < 0.001$), and there was no effect of sex on this relation ($F_{1,42} = 2.45$, $P = 0.13$). When we considered each category of aggressive behaviour separately, both display ($R^2 = 0.33$, $F_{1,42} = 24.91$, $P < 0.001$) and contact aggression (Spearman correlation: $r_S = 0.43$, $N = 45$, $P = 0.003$) were significantly positively correlated between winners and losers. Winners performed more aggressive acts (displays + contact aggression; paired t test: $t_{44} = 7.40$, $P < 0.001$), displays (paired t test: $t_{44} = 4.86$, $P < 0.001$) and contact aggression (Wilcoxon signed-ranks test: $Z = -5.18$, $N = 45$, $P < 0.001$) than did losers.

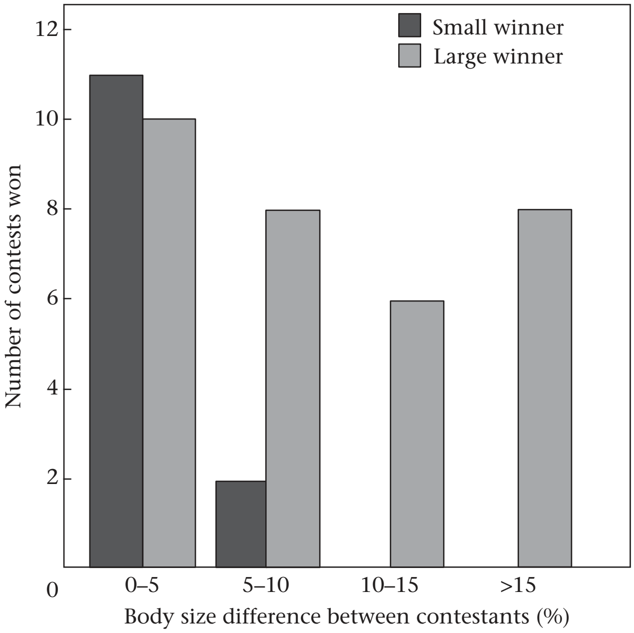

Figure 1. Number of contests won by the larger or the smaller contestant at different levels of body size asymmetry in Tanganyikan cichlids, *Neolamprologus pulcher*.

Display Phase

A period of noncontact display always preceded acts of contact aggression. The display-only phase, measured from the first display of the contest to the first physical contact, lasted an average of 87.89 ± 17.15 s (range 1–510 s). Noncontact displays continued to be used throughout the course of the interaction even after physical contact behaviours were introduced. Therefore, despite the period of display prior to physical contact, the fights did not seem to have a discretely phasic structure. The duration of the display-only phase did not predict the total duration of the contest (ANCOVA: $R^2 = 0.02$, $F_{1,42} = 0.40$, $P = 0.53$). The duration of the display phase was positively correlated with winner SL ($R^2 = 0.12$, $F_{1,42} = 4.46$, $P = 0.04$) but not with loser SL ($R^2 = 0.03$, $F_{1,42} = 0.28$, $P = 0.32$).

Contest Duration

Contests lasted about 10 min on average (mean: 578.4 ± 44.7 s, range 85–1194 s). Male and female contests did not differ in duration (Welch's t test: $t_{40.59} = 0.51$, $P = 0.62$). There was a negative relation between winner size and contest duration (ANCOVA: $R^2 = 0.15$, $F_{1,42} = 7.18$, $P = 0.01$; Fig. 2a), but loser size was not related to contest duration ($R^2 = 0.05$, $F_{1,42} = 2.05$, $P = 0.16$; Fig. 2b) and the sexes did not differ in the above relations ($P > 0.05$).

Contest Intensity

The average contest intensity was 5.6 ± 0.4 aggressive acts/min (range 1.4–13.3 acts/min). This total intensity can be broken up into an average of 4.4 ± 0.3 displays/min (range 0.9–11.0 acts/min) and 1.6 ± 0.2 acts of contact aggression/min (range 0–8.8 acts/min). Male and female contests did not differ in the frequency of aggression (total aggression rate: Welch's t test: $t_{42.6} = 0.17$, $P = 0.86$; display rate: $t_{39.8} = -0.48$, $P = 0.64$; contact aggression rate: $t_{36.4} = 1.08$, $P = 0.29$). Contest duration was negatively correlated with total contest intensity (ANCOVA: $R^2 = 0.25$, $F_{1,42} = 13.81$, $P = 0.001$) and display intensity ($R^2 = 0.20$, $F_{1,42} = 10.12$, $P = 0.003$), but not with contact aggression intensity ($R^2 = 0.08$, $F_{1,42} = 3.80$, $P = 0.06$). There were no sex differences in any of these relationships (all Ps > 0.05).

Total contest intensity was not correlated with winner body size ($R^2 = 0.09$, $F_{1,42} = 0.37$, $P = 0.55$) or loser body size ($R^2 = 0.41$, $F_{1,42} = 1.78$, $P = 0.19$). When the analysis was restricted to contact aggression only, loser size did not correlate with contact aggression intensity ($R^2 = 0.06$, $F_{1,42} = 3.10$, $P = 0.24$); however, winner size and contact aggression intensity were negatively related ($R^2 = 0.13$, $F_{1,42} = 4.92$, $P = 0.03$), so that fights with smaller winners had more intense contact components (more contact aggression per minute). There was no sex difference in this relationship ($F_{1,42} = 1.14$, $P = 0.29$).

DISCUSSION

Contests and conflict are common among members of social groups with dominance hierarchies, as rank is typically determined by aggressive interaction (Drews 1993). Our observations of behaviour within dyadic resource contests in *N. pulcher* may shed light on conflict resolution within social groups.

Contest winners were usually larger than losers. Interestingly, winner but not loser size was negatively correlated with contest duration and contact aggression intensity. None of the assessment models explicitly make this prediction. Larger winners won contests more quickly and with less intense contact aggression. Well-matched contestants had longer fights with more intense aggressive behaviour; however, these results were driven by winner absolute body size not the size differences between winners

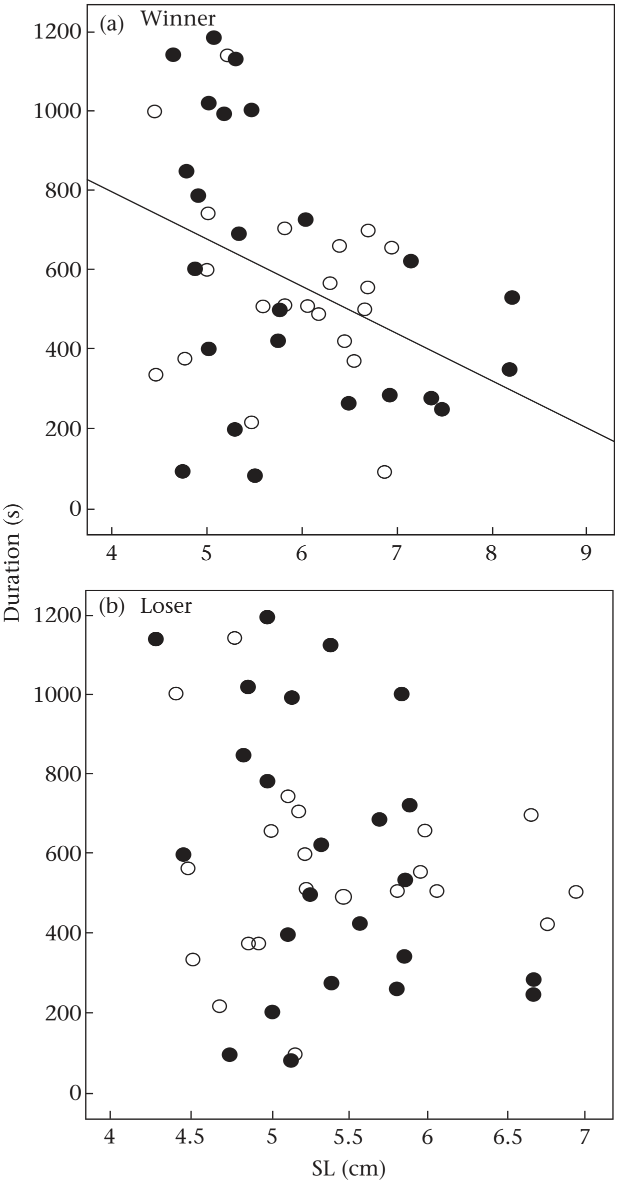

Figure 2. Linear relation between contest duration and (a) winner and (b) loser body size (standard length, SL) in Tanganyikan cichlids, *Neolamprologus pulcher*. Solid circles: males; open circles: females.

and losers. Our results are incompatible with the logic of self-assessment as it appears that *N. pulcher* perceive information about their opponent's RHP and alter behaviour accordingly. Our results are also incompatible with the logic of mutual assessment, where individuals compare opponent and own RHP. It is worth noting that while the cumulative assessment model cannot be conclusively discriminated from mutual assessment model using the current method (both models make similar predictions about winner and loser RHP and contest cost; see Briffa & Elwood 2009), the lack of a relation between loser size and contest duration in our study is inconsistent with the logic of cumulative assessment, where the decision to relent is ultimately based on the loser's own cost threshold (Payne 1998).

In reality, a complex blend of assessment strategies may better describe contest behaviour across taxa (for recent reviews, see: Arnott & Elwood 2009a; Briffa & Sneddon 2010). Contest behaviour that does not conform precisely to any of the existing models has been observed in a variety of taxa (e.g. Jennings et al. 2004; Briffa & Elwood 2002; Kelly 2006; Briffa 2008; Elias et al. 2008; Hsu et al. 2008). Our results are most consistent with opponent assessment without self-assessment. Other studies have found some evidence for opponent-only assessment. For example, Arnott & Elwood (2010) found some indication of opponent-only assessment during contests in another cichlid (*Amatitlania nigrofasciata*), fish with fighting experience showed longer delays to resume fighting following interruption by a simulated predator attack when their opponent was a large fish. Prenter et al. (2008) found that swordtail males (*Xiphophorus helleri*) assess the length of each other's swords and use this information when deciding to persist in a contest, but do not seem to fight with reference to their own sword length. Perhaps most convincingly, Rillich et al. (2007) found that male crickets (*Gryllus bimaculatus*) decide to give up and flee from a contest based primarily on the actions of their opponent. Combined with these previous results, our study suggests that opponent assessment without any form of self-assessment may represent a heretofore under-appreciated mechanism of assessment in animal contests.

Neolamprologus pulcher always displayed prior to making physical contact, but the length of this display-only period was quite variable, and sometimes was extremely brief (minimum observed = 1 s). Displays did not cease once physical contact began and they continued at a high rate until the contest was settled. Lower-cost displays are thought to facilitate opponent assessment (Enquist et al. 1990; Keeley & Grant 1993; Hurd 1997; Reddon & Hurd 2009; Arnott & Elwood 2009b, c, 2010) and appear to be an important component of *N. pulcher* contests.

We found no sex differences in any measured aspect of contest behaviour. Male and female *N. pulcher* contests were of similar length and intensity. Winner RHP appeared to have the same effect on contest duration, intensity and outcome in both sexes. Our study convincingly demonstrates that fight tactics do not differ in any substantive way between the sexes in this species and that male and female aggressive behaviour and contest decision making are indistinguishable. This contrasts with what has been found in some other species (Cole et al. 1980; Holder et al. 1991; Johnsson et al. 2001; Briffa & Dallaway 2007; Draud et al. 2004; Arnott & Elwood 2009b) where males and females fight according to different rules.

Interestingly, the size difference threshold that was usually decisive (~5% difference in SL) is strikingly similar to the size difference observed between rank-adjacent members of naturally occurring *N. pulcher* social groups (Taborsky 1984, 1985). It is possible that *N. pulcher* dominance hierarchies maintain a level of size stratification, either by suppression from dominant members (Taborsky 1984; Wong et al. 2008) or via self-restraint by subordinates (to avoid punishment; Wong et al. 2008), such that each member of the dominance hierarchy can physically dominate its immediate subordinate (Wong et al. 2007). There is some evidence of strategic growth in *N. pulcher* males (Heg et al. 2004) but not in females (Hamilton & Heg 2008; Heg 2010), providing some support for this hypothesis, at least in males. Our results suggest that the size stratification of the dominance hierarchy may be influenced by assessment of groupmate RHP.

The rate of aggression in our staged resource contests was approximately 10-fold higher than the rates of aggression reported among subordinate helpers within a stable *N. pulcher* social group (Wong & Balshine 2010b). In the Wong & Balshine (2010b) study, when a helper was removed from the dominance hierarchy, the ascending fish (that moved into the vacated dominance position) approximately doubled its rate of aggression within the group, resulting in a rate of aggression that was still approximately five-fold lower than the aggression rates we observed during our staged resource contests. Apparently, alterations to the dominance hierarchy in *N. pulcher* are resolved through aggression, but rates of aggression between familiar group members are typically lower than aggression observed in resource contests between strangers. *Neolamprologus pulcher* do naturally engage in escalated contests, for example, during territory take-overs or group-joining events (Balshine-Earn et al. 1998; Stiver et al. 2006; Fitzpatrick et al. 2008), and in these situations we would expect to observe aggression rates closer to what we have observed in the staged contests. Within an established social group, however, individuals appear to constantly engage in low-level aggression rather than punctuated bouts of escalated aggression, although these may occasionally occur. The decision rules that dictate this day-to-day low-level aggression are likely to share a mechanistic basis with the rules used in escalated contests; therefore, the decision-making mechanisms we have described here should help elucidate the nature of aggressive conflict within permanent social groups and conflict resolution within animal societies.

In summary, we found evidence for opponent RHP assessment during staged dyadic resource contests in a cooperatively breeding vertebrate. The evidence suggests that assessment of opponent size appears to be germane to strategic and tactical decision making during *N. pulcher* contests, and is more important than assessment of one's own RHP, a prediction not explicitly made by any of the existing models of contest behaviour. We found no evidence for sex differences in contest behaviour: male and female *N. pulcher* fought by the same rules and made decisions based on the same information, suggesting that the costs and benefits of aggression are similar in males and females of this species. To our knowledge this is the first study to stage controlled contests in a cooperatively breeding vertebrate and to investigate the decision-making process during these conflicts.

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Chapter 4: Probing aggressive motivation during territorial contests in a group-living cichlid fish

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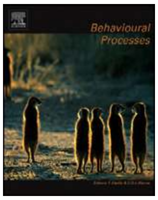

Probing aggressive motivation during territorial contests in a group-living cichlid fish

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ABSTRACT

Many animals fight to win resources, repel competitors or establish dominance in a social group. Mutual-assessment of fighting ability, where competitors gather and compare information about their opponent's as well as their own fighting ability has been the dominant theoretical framework for understanding decision-making during fights. However, self-assessment, where each individual has a cost threshold and fights up until that point, may be more common than previously appreciated. In this study, we attempted to discriminate between these two potential assessment mechanisms in a group-living cichlid fish, *Neolamprologus pulcher* by probing aggressive motivation during a territorial contest. We measured aggressive motivation, and used this metric to investigate assessment rules during an ongoing contest. We predicted that if these social fish use self-assessment, we would observe a positive correlation between the fighting ability of the probed animal and its aggressive motivation. Alternatively, if mutual-assessment is used then we predicted we would find a negative effect of the opponent's fighting ability on the aggressive motivation of the probed fish because fish should be less motivated to fight against formidable opponents. Our results did not support either of these predictions. In contrast we found that small individuals were more aggressively motivated regardless of their opponent's size. We discuss this result in the context of theoretical models of aggression in individuals of small body size.

1. Introduction

Aggressive contests are common when animals resolve conflict by direct interaction (Huntingford and Turner, 1987; Archer, 1988; Maynard-Smith and Harper, 2003; Briffa and Sneddon, 2010). Contests can be costly in terms energy (Briffa and Elwood, 2004; Castro et al., 2006), lost time for feeding and mating (Kemp and Wiklund, 2001), diverted attention from potential predators (Jakobsson et al., 1995), and the risk of injury or death (Enquist and Leimar, 1990). Consequently, contests typically include some form of assessment, which may reduce these costs to one or both competitors (Parker, 1974; Enquist and Leimar, 1983; Arnott and Elwood, 2009a).

Fighting ability (commonly referred to as resource holding potential or power, abbreviated 'RHP' in either case) is a key determinant of fight outcome and dynamics (Parker, 1974; Maynard-Smith, 1982). Models of fighting ability assessment can be broken up into two basic categories: 1) Self-assessment, where animals make the decision to persist or relent in a contest based on a threshold for costs incurred and, 2) mutual-assessment, where each contestant gathers some information about the strength of its opponent and compares that information to its own fighting ability (Maynard-Smith and Parker, 1976; Parker and Rubenstein, 1981; Enquist and Leimar, 1983; Enquist et al., 1990; Mesterton-Gibbons et al., 1996; Payne and Pagel, 1996, 1997; Payne, 1998). Mutual-assessment is more complex, but carries with it the distinct advantage of reducing fight costs for the loser in asymmetric contests (Arnott and Elwood, 2009a).

Perhaps because mutual-assessment is intuitively satisfying, and mirrors the decision-making processes in our own species (Sell et al., 2009, 2010), mutual assessment has become the dominant paradigm in the study of aggression (Taylor and Elwood, 2003; Briffa and Elwood, 2009; Arnott and Elwood, 2009a). The most commonly reported evidence for mutual-assessment is a negative relationship between the degree of asymmetry in fighting ability between the two contestants and the duration of the contest (Taylor and Elwood, 2003). The logic being that closely matched contestants will have greater difficulty determining which one has an advantage, and therefore will need to fight longer before determining which is stronger (Enquist and Leimar, 1983). However, the relationship between fighting ability asymmetry and contest duration can be driven entirely by a positive relationship between the fighting ability of the loser and the duration of the contest

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(Taylor and Elwood, 2003). Therefore self-assessment can produce the same result as mutual-assessment.

Fortunately, Taylor and Elwood (2003) recommend a clever way to distinguish between these two forms of assessment by examining the effects of winner and loser fighting ability on contest duration separately. Under mutual assessment, opposite effects of winner and loser fighting ability on the duration of the contest is expected with stronger losers lengthening the contest and stronger winners shortening it. Under self-assessment, only the loser's fighting ability should be positively related to the duration of the contest (because the fight ends when the loser gives up) whereas the winner's fighting ability should be unimportant. The independent analysis of winner and loser fighting ability has been successfully applied in a number of empirical studies on a wide range of taxa (e.g., shore crabs, *Carcinus maenas*: Smallegange et al., 2007; jumping spiders, *Phidippus clarus*: Elias et al., 2008; house crickets, *Acheta domesticus*: Briffa, 2008; sierra dome spiders, *Neriene litigiosa*: Keil and Watson, 2010; cichlid fish, *Neolamprologus pulcher*: Reddon et al., 2011; green anole lizards, *Anolis carolinensis*: Garcia et al., 2012; fallow deer, *Dama dama*: Jennings et al., 2012).

Recently, Arnott and Elwood (2009a) suggested that assaying aggressive motivation during an ongoing contest might be another way to discriminate between self- and mutual-assessment. The motivational probe technique involves interrupting one of two fighting animals with a simulated predator attack and measuring the latency for this disturbed animal to resume fighting its rival. The duration until resuming the fight can be taken as an inverse metric of aggressive motivation. This assay was initially developed for use during fights in the hermit crab (*Parurus bernhardus*; Elwood et al., 1998; Briffa and Elwood, 2001), but has been subsequently adapted for use during contests in a fish, the convict cichlid (*Amatitlania nigrofasciata*; Arnott and Elwood, 2009b, 2010). The motivational probe assay assumes that the interruption by a novel startle stimulus will activate antipredator responses in the startled animal, and that the motivation to continue fighting will be put in conflict with the motivation to avoid predators (Culshaw and Broom, 1980; Elwood et al., 1998). Presumably, animals that are more motivated to fight will show shorter latencies to resume aggression than those that are less motivated, and thus latency to resume aggression can be taken as an inverse measure of aggressive motivation (Elwood et al., 1998; Arnott and Elwood, 2009a,b, 2010). This method offers several potential advantages over the measures of contest cost taken at the end of a fight (e.g. fight duration, injury or energy expenditure). Namely, contest cost measures taken at the end of a contest can only provide insight into the final decision made by the loser (as the loser decides when the fight is over) while measuring aggressive motivation throughout a contest may provide information about the ongoing decision-making process in both winners and losers.

In this study, we applied the motivational probe technique for the first time to staged resource contests in the group-living cichlid fish, *N. pulcher*. *N. pulcher* are small cichlid fish endemic to Lake Tanganyika, Africa that form permanent social groups consisting of a single dominant breeding pair and 1–20 subordinate non-reproductive adults (Taborsky and Limberger, 1981; Taborsky, 1984, 1985; Balshine-Earn et al., 1998; Balshine et al., 2001; Wong and Balshine, 2011a). The subordinate helpers may be either related or unrelated to the breeding pair and to each other (Stiver et al., 2008). *N. pulcher* groups are organized as strict linear dominance hierarchies that are determined by body size and hence fighting ability (Taborsky, 1984, 1985; Balshine et al., 2001; Wong and Balshine, 2011a,b). Rank in the dominance hierarchy is strongly related to fitness outcomes of *N. pulcher*, and only a few fish ever attain a dominant breeding position (Stiver et al., 2004; Wong and Balshine, 2011a). Dominance relationships are formed and tested by direct aggressive interactions (Arnold and Taborsky, 2010; Riebli et al., 2011), and therefore fighting behaviour in general and the assessment of fighting ability in particular is of paramount importance to the social structure of *N. pulcher* groups (Taborsky, 1984, 1985; Hamilton et al., 2005; Wong and Balshine, 2011b; Reddon et al., 2011).

A previous study on fighting behaviour in *N. pulcher* found that body size asymmetry between the competitors predicted contest duration and that a 5% advantage in body size was sufficient to determine which individual would win (Reddon et al., 2011). However, the results from Reddon et al. (2011) did not fit perfectly with any of the extant contest assessment models. Namely, opponent size was the primary determinant of fight dynamics and the fighting ability of the losing fish did not predict the duration or intensity of contests. In the present study, we aimed to further investigate the aggressive assessment abilities in this highly social fish using the motivational probe technique (Elwood et al., 1998). We predicted that if the latency to resume aggression correlates negatively with the probed individual's fighting ability and positively with its opponent's fighting ability then mutual-assessment likely underlies *N. pulcher* contests. In contrast, if the latency to resume aggression was uncorrelated with opponent fighting ability but negatively related to the probed individual's fighting ability then this would suggest that self-assessment is a better fit. We test these two competing predictions here.

2. Materials and methods

2.1. Animals

The fish used in this study were the laboratory-reared descendents of *N. pulcher* collected from Lake Tanganyika, Africa. The fish lived within naturalistic social groups, housed one group per 189 L glass aquarium (92 × 41 × 50 cm). Each group consisted of a dominant breeding pair and 2–10 adult subordinate helpers. Each group was housed in an aquarium that contained a pair of flowerpot halves to serve as brood chambers, two large foam filters (10 × 10 × 30 cm), and 3 cm of crushed coral sand substrate. Aquaria were maintained at 26 ± 2 °C and exposed to a 14L:10D light cycle. Fish were fed commercial cichlid flake food (Hagen Nutrafin basix) once daily, 6 days per week.

We used 50 (26 males and 24 females) subordinate helper fish from these groups to form 25 experimental pairs. The fish ranged in size from 44.0 to 65.8 mm standard length (SL, measured from the tip of the snout to the caudal peduncle). We also weighed each fish and found that SL and mass were strongly correlated ($r = 0.94$, $N = 50$, $p < 0.0001$), so we chose to use SL for all analyses to be consistent with previous research on contest behaviour in this species (Reddon et al., 2011, 2012). Fish were always paired with an unfamiliar, same sex, individual. Pairs were not size matched and the size asymmetry within each pair ranged from 0.7 to 26.9% different in SL. *N. pulcher* naturally fight with individuals from their own group and with potential group joiners over shelters and to establish dominance rank (Wong and Balshine, 2011a,b; Riebli et al., 2011, 2012; Reddon et al., 2011, 2012).

2.2. Procedure

Contests took place in a 38 L aquarium (50 × 26 × 30 cm). A permanent transparent barrier separated the contest aquarium into two equally sized compartments (25 × 26 × 30 cm) and a pair of fish from two randomly chosen social groups were placed into these two compartments. The pair of fish had no visual contact for a 3 h acclimation period because an opaque, removable barrier was inserted adjacent to the transparent barrier. We chose a 3 h acclimation time because prior work in our lab has suggested that a 3 h residence time is sufficient to induce a robust aggressive response to perceived intruders in *N. pulcher* (Reddon et al., 2012). Following the acclimation period, the opaque barrier was removed (bar one 6 cm strip, that hid the motivational probe from the stimulus fish, see below), and the two fish could begin to interact across the transparent barrier. Fish were allowed to attack each other across the barrier for 2 min. We then probed aggressive motivation of one of the two fish, determined at random beforehand and henceforth referred to as the focal fish. The motivational probe consisted of a 22 g glass marble dropped through a plastic tube into the focal fish's compartment from a height of 26 cm. The marble was dropped with a remote trigger activated by an experimenter from 2.5 m away. The falling marble was visible to the focal but not the stimulus fish. The falling marble created a noticeable splash as it entered the water and sunk down to the substrate. However, the physical disturbance of the water caused by the falling marble did not penetrate the barrier separating the focal compartment from the stimulus fish compartment and appeared to be perceptible only to the focal fish. Following Arnott and Elwood (2009b, 2010), we assumed that this novel visual/tactile disturbance simulated a potential predator attack. The trials were video recorded for a period of 300 s following the marble drop at which point the opaque barrier was reinserted between the fish, terminating the trial. A trained observer, blind to the sex and the body size measurements of the fish, later scored the video recordings. We measured the time it took for the focal fish to resume attacking its opponent (biting at and/or ramming the barrier in the direction of the opponent). Fish that had not resumed aggression within 300 s were assigned a score of 300 s.

We considered the latency to resume physical attacks on the opponent fish as an inverse measure of the focal fish's aggressive motivation (Elwood et al., 1998; Arnott and Elwood, 2009b). Fish that were more motivated to attack their rival, presumably resumed aggression sooner than those that are less motivated to resume fighting. We also measured the time taken to begin attacking initially following the removal of the barrier as an index of aggressive motivation at the beginning of the fight and the time spent frozen motionless following the marble drop as an index of the fish's fear of the marble drop stimulus. We predicted that the motivation to resume fighting following the motivational probe would correlate with the focal fish's perception of its chances of winning and that fish that perceived themselves as having a high probability of winning a fight would be more motivated and would therefore resume fighting sooner.

Each pair was tested twice, 3 h apart with each fish in an experimental pair serving as both the focal fish and the stimulus fish in a randomly determined order. During the 3 h intertrial interval, the opaque barrier was reinserted between the fish preventing visual contact, and the marble dropping apparatus was shifted over to the other compartment of the contest aquarium.

2.3. Data analysis

We analyzed the time taken to attack from the beginning of the removal of the opaque barrier, the time spent frozen after the marble drop and the latency to resume fighting after the marble drop using generalized linear mixed models (GLMM) with an identity link function treating each pair as an experimental unit (following Briffa and Elwood, 2010). We included testing order as a within experimental units factor, sex as a between experimental units factor and both focal and opponent SL as continuous covariates. Analysis was conducted using SPSS 20.0 (SPSS Inc., Chicago, IL, USA).

2.4. Ethical note

Fish in this experiment did not suffer any injury nor did they exhibit any signs of undue stress. The methods for animal housing, handling and experimental protocols were assessed and approved by the Animal Research Ethics Board of McMaster University (Animal Utilization Protocol #10-11-71) and adhere to the guidelines of the Canadian Council for Animal Care and the Animal Behaviour Society.

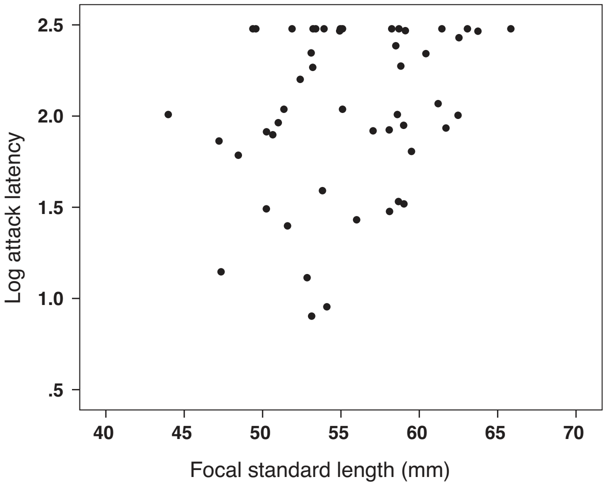

Fig. 1. Focal size (standard length in mm) plotted against the log of the latency to resume aggression following an experimental disturbance ($p = 0.001$).

3. Results

Fish took an average of 63.6 ± 13.0 s to attack their rival across the transparent barrier following the removal of the opaque barrier. There was no difference between males and females in the time taken to attack rivals (GLMM effect of sex: $F_{1,45} = 0.13$, $p = 0.72$) or between fish in the first versus the second trial (effect of test order: $F_{1,45} = 0.40$, $p = 0.53$). There was no statistically significant relationship between the size of the focal fish or its opponent's size and the latency to attack after the barrier had been raised (effect of focal SL: $F_{1,45} = 0.05$, $p = 0.83$; effect of opponent SL: $F_{1,45} = 2.00$, $p = 0.16$).

Following the marble drop, on average, the focal fish remained frozen motionless for a period of 21.7 ± 5.3 s. Males and females froze for a similar amount of time (GLMM effect of sex: $F_{1,45} = 1.90$, $p = 0.18$) and there was no effect of testing order on the duration that fish remained frozen (effect of testing order: $F_{1,45} = 2.90$, $p = 0.10$). The body size of the focal fish was not significantly related to its freezing duration (effect of focal SL: $F_{1,45} = 0.06$, $p = 0.81$) nor was the size of its opponent (effect of opponent SL: $F_{1,45} = 0.85$, $p = 0.36$).

Fish took an average of 158.5 ± 16.0 s to resume attacking their opponent following the motivational probe. The size of the focal fish was positively related to its latency to resume attack (GLMM effect of focal SL: $F_{1,45} = 11.45$, $p = 0.001$; Fig. 1) such that smaller fish were faster to resume aggression than were larger fish. There was no relationship between opponent size and the latency of the focal fish to resume attacking (effect of opponent SL: $F_{1,45} = 0.54$, $p = 0.47$; Fig. 2). Also, there was no difference between males and females in terms of their latency to resume aggression (effect of sex: $F_{1,45} = 0.86$, $p = 0.36$) nor was there any effect of testing order (effect of test order: $F_{1,45} = 2.30$, $p = 0.14$). The latency to resume attacking following the probe stimulus was not significantly related to the latency to begin attacking initially following the removal of the opaque barrier ($F_{1,46} = 0.28$, $p = 0.60$) or the time spent frozen following the marble drop ($F_{1,46} = 1.25$, $p = 0.27$).

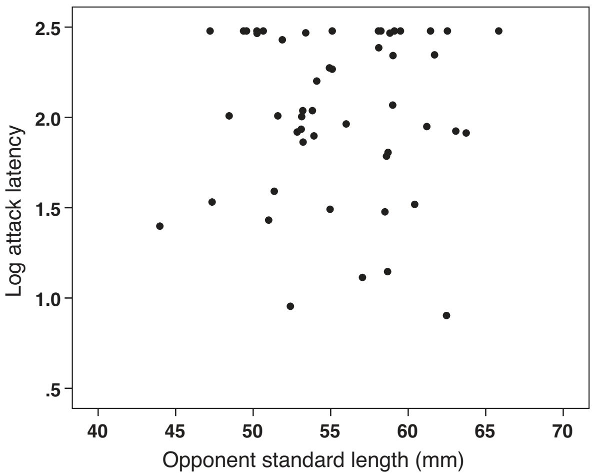

Fig. 2. Opponent size (standard length in mm) plotted against the log of the latency to resume aggression following an experimental disturbance ($p = 0.47$).

4. Discussion

Smaller individuals were more, not less, motivated to resume aggression and opponent size had no influence on the latency to resume aggression. Neither focal nor opponent size was significantly related to either the initial latency to begin aggression after the opaque barrier was raised or the time spent frozen following the marble drop. Therefore, our study does not provide clear evidence for either self- or mutual-assessment. Note that the only previous study to use a motivational probe to investigate assessment during resource contests, also did not find unambiguous support for either set of models (Arnott and Elwood, 2010). We did not find any effect of testing order on any of our measures, suggesting that there was not a priming or a habituation effect on aggression in *N. pulcher* with the intertrial interval used here. Males and females did not differ in their latency to attack initially or following the marble drop and spent equal time frozen after the marble drop.

Heightened aggressivity among small individuals has been observed in other species of fish and invertebrates (e.g. Dow et al., 1976; Enquist and Jakobsson, 1986; Ribowski and Franck, 1993; Smith et al., 1994; Morris et al., 1995; Moretz, 2003; Reddon and Hurd, 2009) and there have been several theories and models proposed to explain these seemingly illogical results (e.g. Grafen, 1987; Dugatkin and Ohlsen, 1990; Bradbury and Vehrencamp, 1998; Just and Morris, 2003; Morrell et al., 2005; Just et al., 2007). The best known of these ideas is the desperado hypothesis (Grafen, 1987) which postulates that weak or poor-quality individuals may have nothing to lose from escalating conflicts over resources they are not likely to secure otherwise. Furthermore, a low-value resource may be worth more to a weak individual than a strong one and hence a weak individual may be more motivated in a contest than is a strong one. The desperado effect is unlikely to explain our observations in *N. pulcher* because, like most fish species, this cichlid grows throughout its lifespan. So an individual with low current fighting ability may eventually become a strong competitor. In fact, the social system of *N. pulcher* is based on queuing within a social group to attain dominant status in the future (Taborsky and Limberger, 1981; Balshine-Earn et al., 1998; Wong and Balshine, 2011a,b). Furthermore, social rank and/or territory are likely to be valued even more by a large individual who might be close to capitalizing on its position and achieving breeding status (Wong and Balshine, 2011b). *N. pulcher* are more likely to play a wait-and-grow strategy as a social subordinate rather than recklessly challenging for dominance as expected by the desperado hypothesis.

An alternative explanation for the heightened aggression in smaller individuals is known as the Napoleon strategy (Morrell et al., 2005). Smaller individuals may be aggressive when the odds in the fight are only slightly against them, the value of the resource is high relative to the costs of fighting and fighting ability is not a perfect determinant of fight outcome (i.e., there are occasional upsets). The Napoleon strategy may be a reasonable explanation for why small *N. pulcher* are more aggressive because both territories and social rank are extremely valuable resources (Balshine et al., 2001; Wong and Balshine, 2011a,b), contests rarely result in severe injuries or death (Reddon et al., 2011) and smaller individuals occasionally win in closely matched contests (Reddon et al., 2011).

The increased aggressive motivation we observed among small *N. pulcher* may be unrelated to any form of fighting ability assessment, for example, small fish may have a higher resting metabolic rate which leads them to behave in a bolder and more aggressive manner (Biro and Stamps, 2010). However, the lack of correlation between the latency to begin the contest and to resume fighting following the startle stimulus suggests that boldness was not an important mediator of aggressive motivation in our study. Nevertheless, it is possible that factors unrelated to assessment may underlie some of the variation in aggression in *N. pulcher* and the precise reasons for the heightened aggressive motivation that we observed in smaller *N. pulcher* will need to be elucidated by future study.

The current experiment employed a motivational probe technique to study aggressive motivation in contesting *N. pulcher* in an attempt to clarify the processes underlying assessment and decision-making in this highly social vertebrate. We found that smaller *N. pulcher* appear to more motivated to resume aggression, but did not find support for any of the extant models of fighting ability assessment.

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Chapter 5: Effects of isotocin on social responses in a cooperatively breeding fish

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Effects of isotocin on social responses in a cooperatively breeding fish

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Oxytocin and its nonmammalian homologues play an important role in modulating a diverse array of social behaviours. Recently, it has been suggested that one of the key functions of oxytocin is to direct attention towards socially relevant stimuli, increase social motivation and guide social decision making. Here, we test whether an exogenous increase in isotocin (the teleost homologue of oxytocin) increases the response to social information in a cooperative breeder, the highly social cichlid fish, *Neolamprologus pulcher*. In our first experiment (a simulated territorial contest), we found that *N. pulcher* injected with isotocin were more sensitive to the size of their opponent regardless of whether their opponent was a live rival or a mirror image. Isotocin-treated fish fought in accordance with the size of their opponent whereas control fish fought according to their intrinsic aggressive propensity. In our second experiment (a social group context), we found that isotocin-treated *N. pulcher* were more responsive to aggressive feedback and produced more submissive displays (an important social signal in this species). These experiments provide evidence that isotocin increases responsiveness to social information and further support the function of the oxytocin family of nonapeptides as a highly conserved regulator of social behaviour across vertebrates.

The evolution of sociality represents one of the most enduring and important questions in behavioural biology (Székely et al. 2010). Why do some species show complex social behaviour, while other closely related species living in similar ecologies spend the majority of their lives in solitude? To answer this question, it is crucial that we develop an integrative perspective on social behaviour that includes a thorough understanding of the proximate mechanisms that generate social behaviour (Insel & Fernald 2004; Young 2009; Soares et al. 2010). The nonapeptide oxytocin (and its nonmammalian homologues; e.g. isotocin in teleost fish, mesotocin in birds and reptiles) represents a promising candidate system for the modulation of social behaviour (for recent reviews see: Donaldson & Young 2008; Goodson 2008; Lee et al. 2009; Ross & Young 2009; Goodson & Thompson 2010; Insel 2010).

Oxytocin acts both as a central neuromodulator and a peripheral hormone (Lee et al. 2009). In the periphery, oxytocin is involved in parturition and milk letdown (Lee et al. 2009). Centrally, oxytocin is essential for the regulation of behaviours related to reproduction, including pair bonding and parental care (Insel & Young 2001). A growing body of research has linked variation in oxytocin and its receptor to social behaviours outside of the realm of reproduction, including affiliation, attachment, trust, generosity, the formation of social memories and the suppression of social anxiety (MacDonald & MacDonald 2010). Taken together, this research suggests that the oxytocin system may be a very general mechanism involved in the regulation of social behaviour (Ross & Young 2009; Goodson & Thompson 2010).

The oxytocin system is highly pleiotropic, affecting an impressive diversity of behaviours across functional contexts (e.g. parental care, cooperation, aggregation, anxiety and aggression). One possible explanation for this functional diversity is that oxytocin may be centrally involved in a higher-order regulatory system with downstream effects on a wide variety of social behaviours transcending functional context (Ross & Young 2009; O'Connell & Hofmann 2011). Recently, a unifying principle has been proposed for the function of oxytocin as a central modulator of attention to social stimuli (Ross & Young 2009). Individuals or species with greater expression of oxytocin (higher circulating levels and/or greater receptor density) may be more attentive to socially relevant stimuli and as a result may be more socially motivated. In support of this idea, it seems that the effects of oxytocin manipulations are specific to explicitly social contexts, while other functionally similar but nonsocial behaviours remain unaffected (Nelson & Panksepp 1996; Ferguson et al. 2000; MacDonald & MacDonald 2010). For example, Kosfeld et al. (2005) found that humans treated with exogenous oxytocin were more accepting of risk in a socially framed economic game (which the authors interpreted as

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increased trust) but not so in a mathematically identical, but nonsocial, version of the game. In animal models, oxytocin suppresses fear associated with social interactions and activates reward centres in the brain (Insel & Shapiro 1992; Insel & Young 2001). Oxytocin appears to be important in the evaluation of the salience and valence of social stimuli, and thus is emerging as a key element of the neural machinery for social decision making (O'Connell & Hofmann 2011). Previous work in nonmammalian vertebrates suggests that the social functions of oxytocin may be evolutionarily ancient (Goodson et al. 2009). Thompson & Walton (2004) found that treatment with exogenous isotocin increased sociability in goldfish, consistent with a role for isotocin in increasing social motivation and interest in social stimuli. Similarly, Braida et al. (2012) found that zebrafish showed greater social motivation after treatment with isotocin. The characterization of the central function of oxytocin as a highly conserved and general regulator of attention to social stimuli and hence social motivation is intuitively satisfying in that it unifies many diverse findings on the social functions of oxytocin into a single conceptual framework.

In the current paper we set out to explore the role of the teleost oxytocin homologue, isotocin (IT), in the social behaviour of the cooperatively breeding cichlid fish, *Neolamprologus pulcher* (also known as *Neolamprologus brichardi*; Duftner et al. 2007). *Neolamprologus pulcher* is a small freshwater fish endemic to Lake Tanganyika, Africa, that forms permanent social groups containing a single dominant breeding pair and several (1–20) adult subordinate helpers (Taborsky & Limberger 1981; Balshine-Earn et al. 1998; Balshine et al. 2001; Heg et al. 2005; Wong & Balshine 2011a). Individual *Neolamprologus pulcher* engage in a rich variety of social behaviours and frequently interact with other members of their own group and with individuals in nearby groups (Taborsky 1984, 1985; Wong & Balshine 2011a). In an experimental context, *N. pulcher* are highly motivated to interact with conspecifics (Jordan et al. 2010; Reddon et al. 2011a). The social complexity of group life observed in *N. pulcher* is highly unusual amongst the fishes and presents an excellent opportunity to test the hypothesis that IT plays a general role in modulating responses to social information.

Here, we report the results of two experiments designed to investigate the role of IT as a regulator of social information use in *N. pulcher*. First, we explored the effects of an experimental increase in IT on behaviour in staged aggressive contests against both mirror images and live rivals. We investigated the general effect of IT on aggression and the effect of IT on opponent assessment (especially, how perceived opponent body size regulates aggression under IT administration compared to control). Opponent body size is an important determinant of contest dynamics in animals (Arnott & Elwood 2009), including *N. pulcher* (Mitchell et al. 2009; Reddon et al. 2011b), and is a vital component of mutual assessment models of contest behaviour, where the relative asymmetry of the contestants is the strongest predictor of fight dynamics and outcome (Parker 1974; Enquist & Leimar 1983; Arnott & Elwood 2009). We predicted that increasing IT would increase sensitivity to social information, and as a result, increase the importance of opponent assessment, thereby strengthening the correlation between opponent fighting ability and focal behaviour. Specifically, we expected that IT-treated fish would be less aggressive against more formidable opponents. In our second experiment, we explored the effects of an experimental increase in IT on social behaviour within a fish's normal social group. In particular, we were interested in the effects of IT on the regulation of aggressive, affiliative and submissive behaviours in permanent social groups where responses to social feedback from other group members are an important part of an individual's daily life. We predicted that experimentally increasing IT would increase responsiveness to social feedback from other group members, resulting in more dramatic responses to pro- and antisocial acts received from group members. Together, these two experiments increase our understanding of the role of IT as a regulator of social information use in a highly social, nonmammalian vertebrate.

GENERAL METHODS

Subjects and Housing

We used 55 adult *N. pulcher* (27 males and 28 females) in these experiments. Experimental fish were all laboratory-reared descendants of wild-caught fish. Each fish was used only once. The fish used in experiment 1 were housed in one of two 527-litre, mixed-sex communal aquaria (183 × 48 × 60 cm) prior to the experiment. These fish had been randomly assigned to these communal tanks as juveniles after being hatched within the social groups maintained in the laboratory. The fish used in experiment 2 were adult subordinate helpers from long-term social groups maintained in our laboratory. Each social group is housed in a 189-litre (92 × 41 × 50 cm) aquarium and consists of a single dominant breeding pair and several (mean: 10; range 7–15) subordinate adult helpers. Water temperature was maintained at 26 ± 2 °C. All fish were fed six times per week on commercially prepared cichlid flakes.

Dosage and Injections

Fish received intraperitoneal injections of isotocin (IT, 1 μg/g of body mass) dissolved in 0.9% saline and/or a 0.9% saline control. Injection volume was tailored to the mass of the fish (25 μl/g). The IT dose was based on previous nonapeptide research in other species (Propper & Dixon 1997; Semsar et al. 2001; Lema & Nevitt 2004; Santangelo & Bass 2006; Mennigen et al. 2008; Filby et al. 2010) and pilot testing in *N. pulcher* in our laboratory. Experiment 1 was a between-subjects design and each fish received only one of the two treatments (IT or saline control). Experiment 2 was a within-subjects design and each fish received both treatments separated by 7 days.

Ethical Note

The fish showed no adverse effects from the injections and resumed normal behaviour within a minute or two. No fish suffered any detectable injury or mortality as a result of the injections or behavioural testing. Focal fish were marked in experiment 2 with a dorsal fin clip to allow for visual identification. Fish recovered immediately from this procedure and showed no adverse effects from the marking. The methods for animal housing, handling and experimental protocols were assessed and approved by the Animal Research Ethics Board of McMaster University (Animal Utilization Protocol number 10-11-71) and adhere to the guidelines of the Canadian Council for Animal Care and ASAB/ABS Guidelines.

EXPERIMENT 1: TERRITORIAL AGGRESSION

Methods

Thirty-six *N. pulcher* (18 females, 18 males; mean standard length = 51.1 mm, range 39.4–62.8 mm) were used in this experiment. Fish were tested for aggressive tendencies in two contexts, first against their mirror image and then against a live same-sex rival across a transparent barrier. Fish were tested in a 38-litre aquarium divided into two compartments by a pair of barriers, one transparent and one opaque, running down the centre (Fig. 1). The far ends of the aquarium were covered with a mirror hidden from

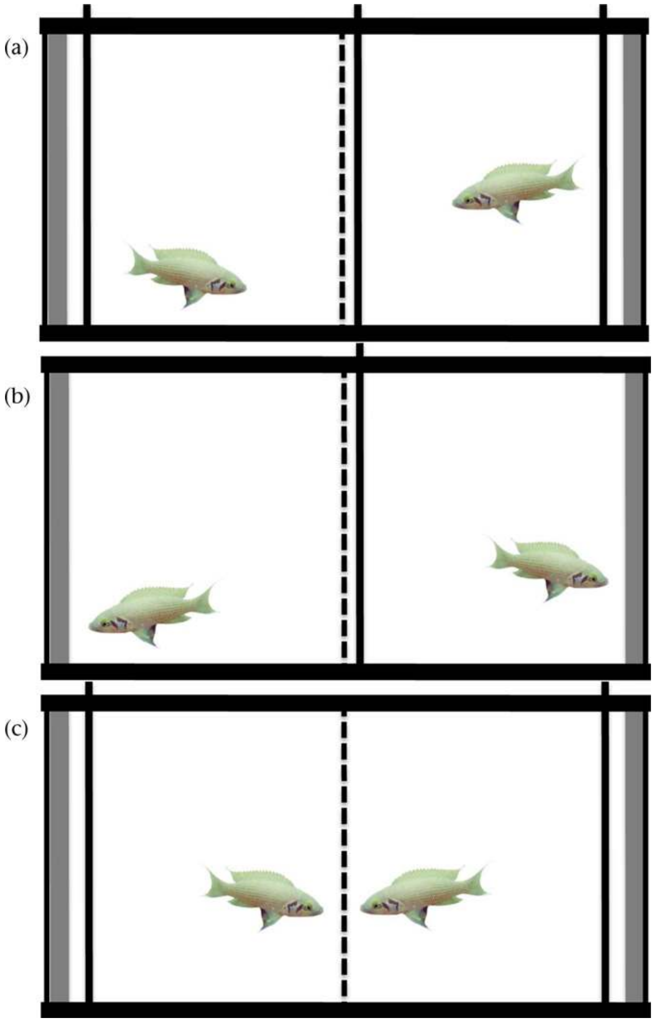

Figure 1. Schematic representation of the territorial aggression testing apparatus used in experiment 1 during the (a) acclimation period, (b) mirror trial and (c) live rival trial. Black line: opaque partitions; broken line: transparent partition; grey line: mirror.

each focal fish by an opaque barrier. We placed a pair of sex-matched fish in each aquarium, one fish in each aquarium half. Individuals were separated from their opponent by the opaque and transparent barriers (Fig. 1a). We allowed each pair of fish a 3 h acclimation period. Aside from sex matching, experimental pairs were chosen randomly, one fish from each of two holding tanks so that all pairs were unfamiliar with one another. The pairs were not size-matched because we wanted to be able to separate statistically the effects of focal and opponent body size on aggression (see Arnott & Elwood 2009). However, the fish were all within the range of asymmetries that would naturally fight (1–12% difference in standard length). There was no significant difference between the average size of IT and saline-treated fish (Welch's test: $t_{33.64} = 0.06$, $P = 0.95$).

We administered an injection of IT to one of the two fish in each experimental pair while the other was given a control injection of saline. Which of the two fish received IT was determined randomly by coin-flip. Fish were given 5 min to recover from the injection and then the barriers covering the mirror were lifted and the fish were allowed to interact with their mirror image for 10 min (Fig. 1b). The barriers were then reinserted, covering up the mirrors, and 1 min later the rival trial began. To do so, we raised the opaque barrier between the two fish, allowing the fish to see and interact with the other pair member (a same-sex fish that had received the opposite treatment) across the transparent barrier for 10 min (Fig. 1c). Both the mirror and the rival trials were videorecorded for subsequent analysis.

The mirror and rival trials were scored from the videorecordings by a trained observer who was blind to the sex and treatment condition of the fish. We assessed the total number of aggressive acts delivered to the mirror and to the rival. We scored the following aggressive behaviours: puffed throats, where the focal fish approaches it opponent with its opercula flared outwards; aggressive head down posture, where the focal fish approaches its opponent with its head angled downward; lateral displays, where the focal fish presents its lateral aspect to its opponent with its head angled downward and/or its unpaired fins held erect; rams, where the focal fish swims quickly towards its opponent and hits its head against the barrier or the mirror but no obvious bite is taken; and bites, where the focal fish makes a biting motion against the barrier or mirror directed towards its opponent (for further descriptions of the behavioural repertoire of this species, see: Taborsky 1984; Buchner et al. 2004; Sopinka et al. 2009). We did not observe any clear escalation of aggressive behaviours (sensu Enquist et al. 1990) in these staged aggressive encounters that lacked physical contact, so we chose to combine all aggressive acts into a single aggression score.

This experiment was analysed using SPSS 20.0 (SPSS Inc., Chicago, IL, U.S.A.) for Macintosh. We computed aggressive acts per minute for each individual's mirror and rival trial and investigated differences between the treatment and control fish. We also examined the correlations between aggressive rates and the standard length (SL) of each fish and the SL of its opponent to determine whether IT affects the use of own and/or opponent size information in *N. pulcher* (Taylor & Elwood 2003). In the case of the mirror trials, the fish's own SL was equal to it perceived opponent's SL (as a fish fighting against a mirror is its own perceived opponent; Rowland 1999; Desjardins & Fernald 2010). Submissive and affiliative acts were rarely observed in this experimental contest context, which is devoid of physical contact between contestants and so these behaviours were not analysed in this first experiment.

We used a linear mixed model (LMM), which treated the contesting pair as the experimental unit (following the recommendation of Briffa & Elwood 2010), with sex as a between-experimental units factor and treatment as a within-experimental units factor to compare aggression rate produced by the IT-treated and the control fish in the live rival trials. Although the two fish could not see one another during the mirror trials, we elected to use a more conservative LMM approach, treating pairs of fish in the same aquarium as a single experimental unit to account for the fact that we could not conclusively exclude the possibility of auditory or olfactory cues being passed across the barriers between the pairs of fish during the time that fish interacted with their mirror images. We included treatment as a within-experimental units factor and sex as a between-experimental units factor in the mirror trials.

We used GLM models, treating body size of the focal fish and its opponent as continuous predictors, to examine the relationship between self and opponent body size and aggression rate for each of the two treatments in the live rival trials (following Taylor & Elwood 2003). We chose not to use a composite measure of the asymmetry between the two contestants because asymmetry measures can lead to erroneous conclusions when analysing contest data (for thorough discussions of this issue, see: Taylor & Elwood 2003; Arnott & Elwood 2009; Briffa & Elwood 2009; Reddon et al. 2011b). Reddon et al. (2011b) found that opponent size was the strongest predictor of contest duration and intensity in unrestrained *N. pulcher* contests. Therefore, size asymmetry may not be appropriate for analysing *N. pulcher* contests. If the asymmetry in size between the two competitors was an important factor determining the contestants' behaviour, then we would expect to observe a positive effect of own body size on aggression and a negative relationship between opponent body size and aggression (Taylor & Elwood 2003). We used GLM models to explore the relationship between aggression and own body size for each of the two treatment groups during the mirror aggression trials. We also used GLM models to assess whether aggression rate in the mirror trial was a good predictor of aggression in the subsequent live rival trial for each of the two treatments.

One pair did not engage in aggressive behaviour during the live rival trial. Because we could not assign this lack of aggression to one fish or the other, we excluded the data from this trial. The residuals from all of our models did not depart significantly from a normal distribution (all Shapiro–Wilk test: $W < 0.97$, all $P > 0.20$) justifying our use of parametric analyses.

RESULTS

Isotocin-treated and control fish had similar rates of aggression against their mirror images (LMM effect of treatment: $F_{1,31.78} = 0.13$, $P = 0.72$), males and females did not differ in aggression against their mirror image (LMM effect of sex: $F_{1,31.78} = 2.43$, $P = 0.13$) and there was no interaction between sex and treatment on aggression against a mirror (LMM sex*treatment effect: $F_{1,31.78} = 0.29$, $P = 0.60$). Isotocin- and control-treated fish were equally aggressive against a live rival (LMM effect of treatment: $F_{1,26.12} = 1.86$, $P = 0.19$), although females were more aggressive, on average, than males (LMM effect of sex: $F_{1,26.12} = 4.50$, $P = 0.04$). There was no significant interaction between treatment and sex on aggression against a live rival (LMM sex*treatment effect: $F_{1,26.12} = 0.009$, $P = 0.93$).

Contrary to our prediction, IT-treated fish showed a positive correlation between their rates of aggression and their own body size (the size of their perceived opponent) in the mirror trials (GLM effect of SL: $F_{1,16} = 8.30$, $P = 0.01$; Fig. 2a). Fish that received a control injection of saline in the mirror trials showed no correlation between rates of aggression and perceived opponent size (GLM effect of SL: $F_{1,16} = 1.35$, $P = 0.26$; Fig. 2b).

Contrary to our original prediction, isotocin-injected fish also showed more aggression against larger live opponents (GLM effect of opponent SL: $F_{1,16} = 6.14$, $P = 0.03$; Fig. 3a), while control fish showed no such response (GLM effect of opponent SL: $F_{1,16} = 0.14$, $P = 0.72$; Fig. 3b). Aggression rates against a live rival were unrelated to a fish's own body size in the IT-treated fish (GLM effect of SL: $F_{1,16} = 1.16$, $P = 0.62$) or in the saline-injected control fish ($F_{1,16} = 0.14$, $P = 0.72$). Hence, own body size did not correlate with aggressiveness in *N. pulcher*, regardless of isotocin treatment, indicating that competitor size asymmetry was not driving the relationships observed with opponent body size.

In IT-treated fish, the rate of aggression against a mirror and against a live rival was not significantly correlated (GLM effect of mirror aggression: $F_{1,15} = 1.02$, $P = 0.33$; Fig. 4a). However, in control fish, rates of aggression against a mirror and against a live rival were strongly positively correlated (GLM effect of mirror aggression: $F_{1,15} = 10.82$, $P = 0.005$; Fig. 4b), indicating that *N. pulcher* were consistently aggressive across stimuli in the control condition.

EXPERIMENT 2: BEHAVIOUR IN SOCIAL GROUPS

Methods

Each of the 19 fish used in this experiment (10 females, 9 males; mean standard length = 47.5 mm, range 40.1–56.8 mm) were given an IT and a control saline treatment, 1 week apart. The order of treatment was counterbalanced across subjects. Only one fish per social group was used in this experiment, and all observations took place within the focal fishes established social groups in their home tank. Focal animals were all mid-ranked helpers (mean rank = 6.5, range 5–8) with an average group size of 10 adults (range 7–15). We chose to concentrate on mid-ranked helpers because these fish produce aggression against other group members ranked below them and they receive aggression from those ranked above. All fish were weighed, measured, individually marked by fin clips and sexed by examination of their external genitalia 1 week prior to the onset of experimentation.

Each fish was observed for 10 min prior to each injection. We counted all aggressive behaviour (see Experiment 1), submissive behaviour (submissive postures, where one fish raises its head upwards towards another, presenting its ventral aspect to the other fish; submissive displays, during which the focal fish assumes a submissive posture and performs a quivering motion with its tail or whole body) and affiliative behaviour (soft touches, where one fish touches the other gently with its head; parallel swims, where

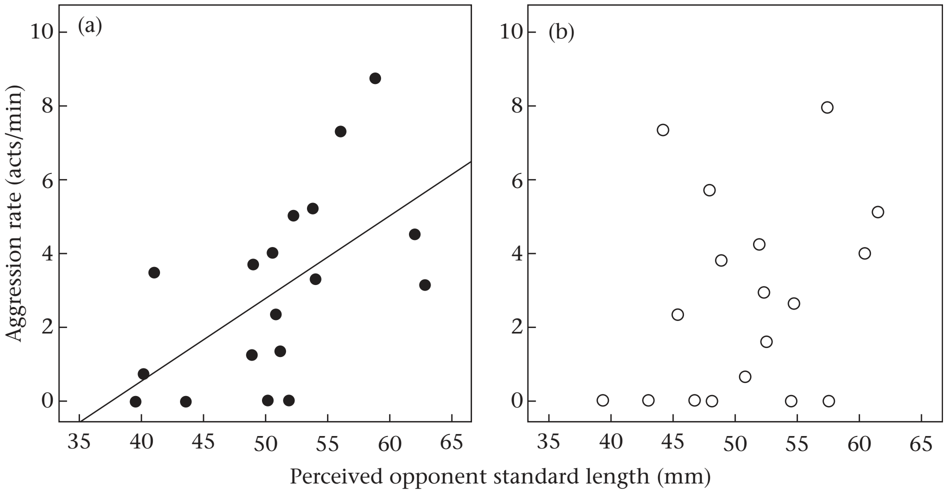

Figure 2. Relation between aggression rate and body size during mirror image aggression trials for (a) isotocin (IT)-treated *N. pulcher* ($P = 0.01$) and (b) saline-treated control fish ($P = 0.26$).

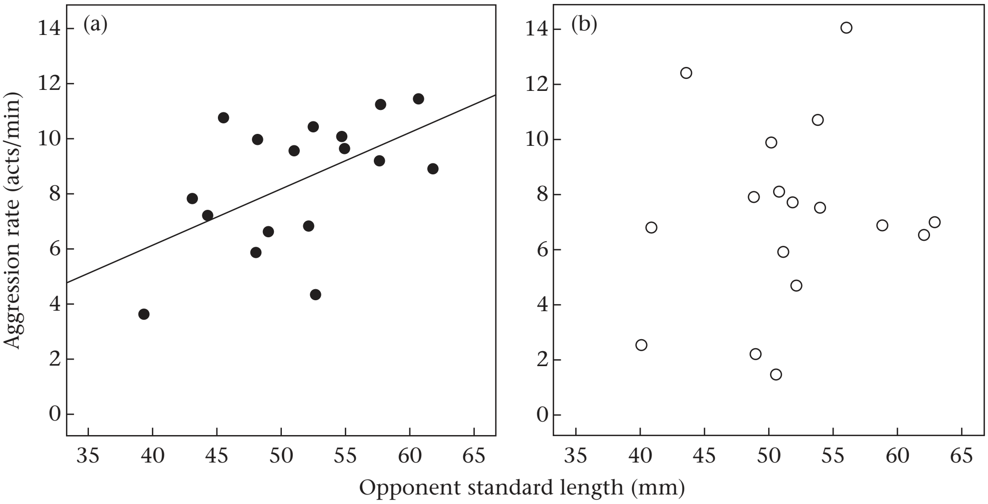

Figure 3. Relation between aggression rate and opponent body size for (a) isotocin (IT)-treated *N. pulcher* ($P = 0.03$) and (b) saline-treated control fish ($P = 0.72$).

two fish swim closely together side by side; follows, where the signalling fish swims closely behind another group member), both given by and received by the focal fish (for a detailed ethogram for this species, see Sopinka et al. 2009). We counted aggressive and affiliative behaviours as the number of these acts produced by the focal fish in 10 min. We calculated the number of submissive behaviours produced per aggressive acts received because submission is most often produced in response to an aggressive act received directly from another conspecific. Following the 10 min pre-injection observation period, we quickly captured the focal fish and injected it with either IT or a saline control. The experimenter delivering the injection and observing the fish was blind to the substance being injected. After the injection, the focal fish was allowed 5 min to recover from handling and injection within its social group. We then observed the focal fish for a 10 min post-injection period during which we recorded the same behavioural measures as in the pre-injection observation. One week later, each focal fish was given the other injection using the same procedure (10 min pre- and post-injection observations). For each of the two injections we calculated the change in the behaviour (aggressive, submissive or affiliative) of the focal fish as follows: (behaviour after injection) − (behaviour before injection), and then compared these values between the IT and control treatments. We also compared the pre- versus post-injection change in total number of behaviours produced across classes of behaviour between the IT and control treatments as a measure of change in total activity level.

We used generalized linear mixed models (GLMM) with an identity link function to analyse this experiment. We included treatment as a within-subjects fixed effect, sex as a between-subjects fixed effect and subject identity as a random effect. This experiment was analysed using SPSS 20.0 for Macintosh.

RESULTS

Fish showed greater submission when they were treated with IT compared to saline (GLMM effect of treatment: $F_{1,35} = 7.05$, $P = 0.01$; Fig. 5a). The fish showed no significant change in their level of aggressive or affiliative behaviour when treated with IT compared to control (aggression: $F_{1,35} = 2.15$, $P = 0.15$; Fig. 5b; affiliation: $F_{1,35} = 0.34$, $P = 0.53$; Fig. 5c). There was no change in overall activity following IT compared to the control treatments ($F_{1,35} = 0.03$, $P = 0.87$; Fig. 5d) and no effect of sex (all $P > 0.05$) in this experiment.

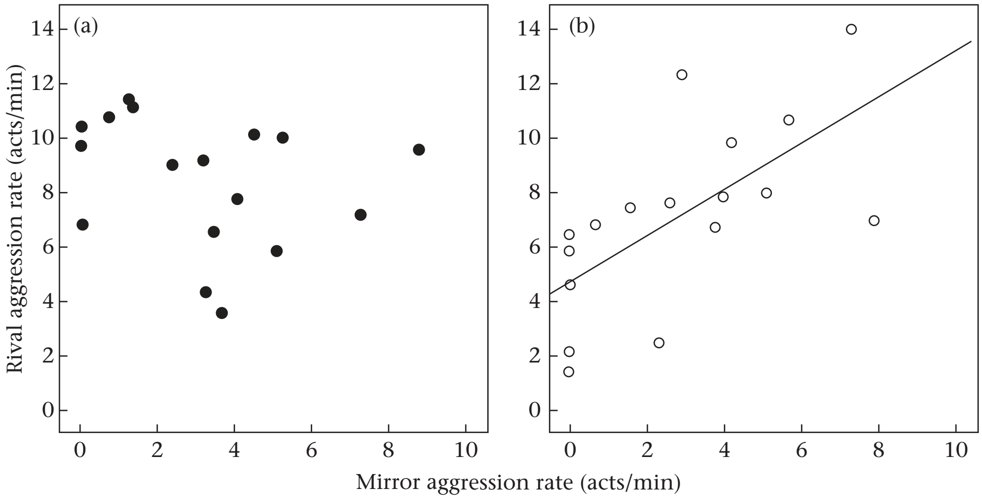

Figure 4. Relation between aggression rate in live rival territorial contest trials and the mirror image aggression trials for (a) isotocin (IT)-treated *N. pulcher* ($P = 0.33$) and (b) control fish ($P = 0.005$).

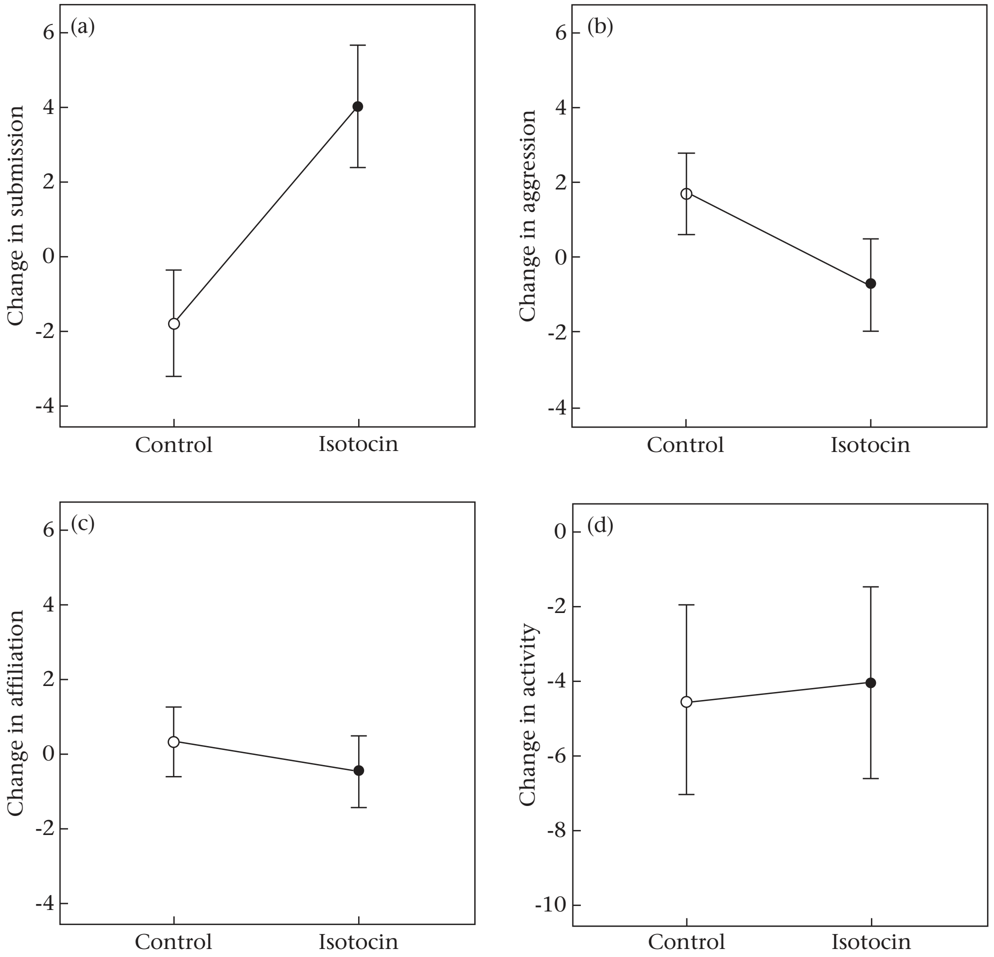

Figure 5. Change in (a) submission ($P = 0.01$), (b) aggression ($P = 0.15$), (c) affiliation ($P = 0.53$) and (d) activity ($P = 0.87$) following injections of isotocin (IT) and saline (control) in *N. pulcher* within their social group.

DISCUSSION

Here we present results of two controlled experiments representing two social contexts, which collectively suggest that experimental increases in IT level enhances sensitivity to social stimuli. In our first experiment we found that *N. pulcher* injected with IT were more sensitive to the size of their perceived opponent and were more aggressive when facing a large opponent. IT-treated fish appeared to make fighting decisions in accordance with their opponents' perceived competitive ability (which is well indicated by body size in this species; Reddon et al. 2011b). In contrast, control fish behaved in accordance with their own intrinsic aggressivity. Aggressive rates for control fish were correlated across the mirror and the live rival trials. Consistent with these results, previous research has also indicated cross-contextual consistency (Riebli et al. 2011) and temporal stability (Chervet et al. 2011) in aggressiveness in unmanipulated *N. pulcher*.

There are at least two reasons why aggression in IT-treated fish is best explained by increased assessment of their perceived opponent (their mirror image) rather than by knowledge of their own fighting abilities. First, there was no correlation between aggression and a given fish's own body size during the rival trials, only a correlation between aggression and opponent body size. It seems logical that the same assessment mechanisms would be in play in a fight against a mirror image and a live rival. Second, previously we showed that assessment of opponent strength is an important determinant of *N. pulcher* contest dynamics, whereas assessment of own strength is relatively unimportant (Reddon et al. 2011b). Although we cannot definitively rule out the possibility that the behavioural effects we observed were driven by the behaviour of the saline-injected opponent and not by the treatment itself, the convergent evidence from the mirror assay (showing the same pattern) suggests this interpretation is most parsimonious.

It is possible that the mirror trial affected the behaviour observed in the rival trial, perhaps by priming the fish to be more aggressive against the rival. However, given that all fish in the aggression experiment received the two assays in the same order, the sequence effects could not have driven the differences we found between the treatments.

Contrary to our prediction, increased opponent body size was correlated positively with increased aggressive behaviour in IT-treated fish during our territorial contest trials. While Reddon et al. (2011b) found that opponent size was the strongest factor influencing the decision to relent in *N. pulcher* contests, large opponent body size was associated with faster acquiescence times and reduced aggressive intensity. Importantly, in those experiments, fish had full physical access (Reddon et al. 2011b), while the fish in the current study were limited to visual displays and noncontact interactions only. It is possible that visual information acts as an 'approach' signal in these fish, while tactile feedback from their opponents provides a 'withdraw' signal (as has been shown in other species; e.g. Rillich et al. 2007). If this is the case, then we would expect that in fights with physical contact, IT-treated fish would approach faster but also relent faster and show less aggression overall than control fish fighting against large opponents. Visual signals need not necessarily motivate approach and can result in withdrawal responses in many species (Hurd & Enquist 2001), so this prediction will need to be carefully tested in a future study. Nevertheless, our results do show that IT-treated *N. pulcher* are more sensitive to opponent body size than are saline-treated control fish, suggesting that this neuropeptide may be important in opponent assessment and contest decision making.

In our second experiment, fish treated with IT showed increased submission when challenged aggressively. This was a specific change in behaviour, as levels of aggression, affiliation and activity remained unchanged. Submissive displays are an important social signal in *N. pulcher* thought to appease dominant group members, stabilize the social hierarchy and reduce the probability of eviction (Bergmüller & Taborsky 2005; Wong & Balshine 2011a, b). Increased submission rates suggest that IT enhancement results in greater sensitivity to within-group conflict and to the social hierarchy in general. Early life social experiences could have an important organizational effect on the isotocin system and result in life-long behavioural variation in *N. pulcher* (Arnold & Taborsky 2010; Taborsky et al. 2012). Taken together, our results provide evidence of increased social sensitivity in fish that experience an experimental increase in an oxytocin homologue and, therefore, provide support for the hypothesis that oxytocin acts to increase the salience of social stimuli (Ross & Young 2009; Soares et al. 2010; O'Connell & Hofmann 2011).

Interestingly, our injections were peripheral and yet the behavioural changes observed were consistent with a central effect. Traditionally, the effects of oxytocin on behaviour have been revealed by using central administrations or peripherally administered blockers with high transmission into the brain (Thompson & Walton 2004; Goodson et al. 2009; Lukas et al. 2011; Oldfield & Hofmann 2011). However, our study joins a growing literature showing that peripheral nonapeptide administration can lead to behavioural changes (e.g. Propper & Dixon 1997; Semsar et al. 2001; Lema & Nevitt 2004; Ring et al. 2006; Santangelo & Bass 2006; Mennigen et al. 2008; Filby et al. 2010; Madden & Clutton-Brock 2011; Braida et al. 2012). There are at least two possible explanations for how our peripherally administered isotocin could have had centrally mediated effects. First, peripheral administrations of oxytocin might penetrate the blood–brain barrier and reach central receptors (Banks & Kastin 1985a, b; Ring et al. 2006). In male mice, peripheral oxytocin injections had behavioural effects and, surprisingly, these could be blocked by central infusions of an oxytocin antagonist, suggesting that the effects of peripheral oxytocin administrations on behaviour are mediated directly by their action on central receptors (Ring et al. 2006). Furthermore, the blood–brain barrier may be much more permeable to neuropeptides in fish than in mammals (Bernstein & Streicher 1965; Olson et al. 1978). Second, the effects we observed may in fact be mediated by action of peripheral receptors (Goodson & Thompson 2010), as the same populations of neurons in the brain may be part of both the central and the peripheral nonapeptide systems, suggesting a tight integration of the central and peripheral actions of nonapeptides (Ross & Young 2009; Ross et al. 2009; Goodson & Thompson 2010). It is therefore possible that exogenous IT binds to peripheral receptors that exert a secondary effect on behaviour through central IT production or some other mechanism. Although we did not measure isotocin levels in the blood or brain, the behavioural effects we observed coupled with prior results using similar doses of nonapeptides in other fish species (e.g. Semsar et al. 2001; Santangelo & Bass 2006; Mennigen et al. 2008; Filby et al. 2010) suggest that our treatment was appropriate.

Each of our two experiments has interesting implications for the role of oxytocin and its homologues in the architecture of social behaviour, and the results of each experiment suggest important follow-up studies. The results of our territorial aggression experiment indicate that IT may be important in opponent assessment. If true, then we would expect animals treated with oxytocin/isotocin to assess one another more effectively and as a result, have shorter, less costly contests. Similarly, an oxytocin antagonist should result in reduced sensitivity to social information, impaired assessment and longer, more costly contests. These predictions require testing, but should provide a valuable window into the effects of the oxytocin system in regulating contest behaviour and territorial aggression. Our results suggest that oxytocin may be a key neurobiological mechanism underlying decision making in resource contests, which would be an important contribution to understanding the evolution of fighting behaviour in animals (Arnott & Elwood 2009).

The results of our social group experiment suggest that isotocin may join steroid hormones (Bender et al. 2006; Fitzpatrick et al. 2008; Taves et al. 2009) as an important proximate modulator of the social dominance hierarchy in *N. pulcher*. Increased submission given in response to within-group aggression should increase the linearity and stability of the dominance hierarchy by attenuating conflict and reducing the likelihood of eviction (Bergmüller & Taborsky 2005). If so, then the dominance hierarchy in groups where some or all members have experimentally increased levels of IT ought to be more stable and well defined. Conversely, the experimental reduction of IT levels using an antagonist should destabilize hierarchies, increasing conflict and the likelihood of group member eviction. Such follow-up experiments would provide important insights into the role of the oxytocin system in regulating the structure of hierarchical animal societies.

Together, our results support the hypothesis that the oxytocin system modulates responses to social information. Oxytocin may act in the brain to divert limited attention towards social interactions and away from other nonsocial activities (Ross & Young 2009), and therefore, is germane to the neurobiology of social decision making (O'Connell & Hofmann 2011). If so, then selection acting on the oxytocin system may be a crucial component of the evolution of social complexity, and an increased understanding of the sociobiological functions of oxytocin will lead to a fuller understanding of social evolution (Goodson 2008; Goodson & Thompson 2010). Our results contribute to an expanding literature that demonstrates the highly conserved basic behavioural functions of the oxytocin system throughout the vertebrate taxon.

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Chapter 6: Isotocin and sociality in a cooperatively breeding cichlid fish

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ABSTRACT

The ultimate functions of sociality, or the tendency to live within a conspecific group, are increasingly understood. However, the proximate mechanisms that mediate this behavior are not yet well established. The oxytocin family of nonapeptide hormones is thought to play an important role in regulating social behavior across a wide range of taxa and social contexts. In the current study, we investigated the influence of exogenous administration of oxytocin and an oxytocin receptor antagonist on sociality in a cooperatively breeding fish, *Neolamprologus pulcher*. In our first experiment, contrary to predictions, we found that a high (and a low) dose of peripherally administered exogenous isotocin (the teleost fish homologue of oxytocin) decreased grouping behavior and the motivation to associate with conspecifics in *N. pulcher*, while an intermediate dose had no effect. In our second experiment, we found that, again contrary to predictions, but consistent with the results from the first experiment, a peripheral administration of an isotocin receptor antagonist increased grouping preference in male *N. pulcher*. These results contribute to a growing body of literature suggesting that broad generalizations regarding the function of oxytocin family of nonapeptide hormones are overly simplistic, and specific behavioral effects depend on both the study species and social context.

Keywords: Grouping behavior, oxytocin, nonapeptide, social decision-making, *Neolamprologus pulcher*

1. Introduction:

Sociality, or the tendency for conspecifics to group together in space and time [2,83], is one of the most widely observed forms of social behavior and one of the fundamental building blocks of social complexity [17,40,41 73]. Sociality varies both within and among species [2,10] and is dictated by the tradeoff between the advantages and disadvantages of living in a group [40]. Living in a group may provide inherent benefits such as the dilution of predation risk and shared vigilance [30,2,53,41] but additional advantages may arise from direct social interactions especially in complex social systems [86]. For example, group living facilitates social learning [20,62] and cooperation among group members [3,9,15,31,82]. Sociality may also have considerable disadvantages, including but not limited to increased conspicuousness to predators [11] and greater competition for resources [35]. While considerable progress has been made in understanding the function of sociality [17,40,41], a comprehensive understanding of the causes and consequences of sociality necessitates an integrative perspective including an appreciation for the proximate mechanisms that underlie grouping behavior [22,23,25,27,73].

One promising potential proximate mediator of sociality is the highly conserved nonapeptide hormone oxytocin [14,23,27,28,34,44,70] and its non-mammalian homologues (e.g., isotocin in teleost fishes; mesotocin in amphibians, non-avian reptiles, birds, and some non-eutherian mammals; [33]). Oxytocin is produced primarily in the hypothalamus where it is released throughout the brain and excreted to the periphery via the pituitary gland [53]. Oxytocin has numerous functions both centrally and peripherally [44] and represents an evolutionarily ancient signaling system dating back to a duplication of the vasotocin gene in early-jawed fish [33]. Peripherally, oxytocin is involved in smooth muscle contraction and milk letdown [44]. Centrally, oxytocin appears to modulate a wide variety of behaviors and plays a role in the stress response. In particular, there is growing evidence that oxytocin is a key regulator of social behavior including pair bonding, affiliation, attachment, trust and parental care [14,23,26,27,44,50,70]. More generally, oxytocin may be important in coding the valence and salience of social stimuli, regulating social motivation and attention, and hence is likely a critical element of the social decision-making system [56,63,69,70].

Despite the vast and growing body of literature on the social functions of oxytocin, the number of studies that have explicitly examined its role in modulating sociality are surprisingly few [23]. In general, oxytocin seems to increase sociality in mammals (e.g., [47,49,74]), as does mesotocin in birds (e.g., [22,24,25]). However, many ecological and life history factors influence the function of nonapeptide hormones [23], and given the small number of studies in a restricted number of taxa, it is not currently possible to arrive at general conclusions about the role of oxytocin and its non-mammalian homologues in regulating sociality.

The teleost fish homologue of oxytocin is isotocin (IT), a highly similar nonapeptide in both structure and function [21,33]. While IT has received far less research attention than has oxytocin, existing data suggests that IT plays a role in the regulation of social behavior in fishes similar to the role of other oxytocin-family nonapeptide hormones [21]. For example, in zebrafish (*Danio rerio*), treatment with exogenous IT increased or decreased sociality depending on dose (resulting in an inverted u-shaped dose-response curve; [7]). Thompson and Walton [81] found that exogenous isotocin increases the tendency to approach conspecifics in goldfish (*Carassius auratus*), although only in individuals that showed a low sociality tendency prior to treatment. Convict cichlid fish (*Amatitlania nigrofasciata*) upregulate endogenous production of IT in preparation for parental behavior, and treatment with a specific IT receptor antagonist interferes with parental care behavior [55]. Injection with a non-specific nonapeptide antagonist delayed but did not prevent pair bonding in the convict cichlid, although this result cannot be conclusively attributed to IT as the antagonist used in this study also blocks the closely related vasotocin system [58]. Given that fish make up by far the most species-rich group of vertebrates and there are so few studies looking at the role of IT in regulating social behavior, more studies in a greater diversity of fish species are warranted.

Neolamprologus pulcher is a cooperatively breeding cichlid fish endemic to Lake Tanganyika, East Africa [39]. *N. pulcher* exhibit a remarkably complex social system and demonstrate an impressive diversity of social behaviors and communicative signals [4,5,13,78,79,84,85]. *N. pulcher* groups consist of a single dominant breeding pair along with 1-20 non-breeding subordinates, including both individuals from previous reproductive bouts and immigrants from other social groups [32,75,76,84]. Subordinate group members may actively assist the breeding pair in their reproductive efforts, serving as helpers-at-the-nest by joining in broodcare, territory maintenance and defence [4,78-80,84,86-88]. *N. pulcher* have recently emerged as a promising model system for the integrative study of social behavior both because of their highly social nature and because they are small bodied, short-lived, and highly amenable to both controlled laboratory experimentation and field study in their natural habitat [84]. Previous work on sociality in *N. pulcher* has shown that *N. pulcher* are highly motivated to associate with conspecifics [13,36,64]. *N. pulcher* prefer to associate with relatives over non-relatives [42], familiar social partners to unfamiliar ones, and prefer large-bodied group mates to small ones [36]. Male *N. pulcher* strongly and consistently prefer to join with large groups over small ones, whereas females consider their social rank when deciding which group to join, preferring to join large groups only when they can join at a high rank [64]. One previous study examined the effects of IT manipulations on *N. pulcher* behavior [63] and found that exogenous IT increased sensitivity to social information. Specifically, Reddon et al. [63] found that *N. pulcher* treated with exogenous IT were more attentive to the characteristics of their opponent during an aggressive interaction and more responsive to aggressive social challenges from dominant individuals within their social groups.

In the current study, we explored the role of IT in modulating sociality in *N. pulcher*. Specifically, we conducted a pair of controlled laboratory experiments manipulating the IT system to determine if this nonapeptide hormone exerts a modulating influence on social motivation in this highly social species. In the first experiment, we gave individual *N. pulcher* an injection of IT at one of three different doses or a vehicle-only control injection, and then provided the injected fish with a choice between interacting with a single stimulus fish or with a group of three stimulus fish. We predicted that an experimental increase of isotocin would augment social motivation in *N. pulcher* and hence would result in fish spending more time associating with the larger group and that there would be more social interactions with increased IT compared to the control treatment. In a second experiment we examined whether endogenous isotocin was playing a role in modulating sociality in *N. pulcher* by injecting study animals with one of three doses of a selective isotocin receptor antagonist (ITA) that has been shown to alter behavior in other fish species, or a vehicle-only control, and then subjecting them to the same behavioral test as in the first experiment. We predicted that the receptor antagonist would interfere with the function of isotocin by competitively blocking the isotocin receptor and hence would decrease social motivation resulting in a reduced sociality response. Based on previous research [64], we made the further prediction that males in both experiments would show a stronger preference to associate with the larger stimulus group than would females. We did not have a specific prediction for how our IT or ITA manipulations would affect the previously demonstrated sex difference in the strength of preference for large groups. Sex differences in nonapeptide effects are commonly reported (e.g., [29,38,47,48]), however, previous research on IT in *N. pulcher* did not uncover any sex-specific effects [63].

2. Experiment 1 - exogenous isotocin:

2.1. Methods

2.1.1. Study animals

The fish used in this experiment were laboratory-reared descendants of *Neolamprologus pulcher* collected from Lake Tanganyika, Africa in 2002 and 2008. Focal fish were housed in a mixed-sex 183 x 48 x 60 cm (527L) communal aquarium. The housing aquarium contained a two-centimeter layer of coral sand substrate, three water filters, two electric heaters, and one thermometer. A total of 50 stimulus fish were also housed in a separate but identical communal aquarium. Fish were fed daily with dried prepared cichlid food. Water temperature was held constant at 26 ± 2º C and within chemical parameters that mimic the natural habitat of *N. pulcher*. Focal fish were always ≥3.5 cm standard length (SL, measured from the tip of the mouth to the caudal peduncle) because *N. pulcher* of this size are sexually mature and can be sexed by examination of their external genitalia [77,78].

2.1.2. Testing apparatus

We tested the focal fish in a social choice apparatus [64] consisting of two 40 x 20 x 25 cm (20 L) stimulus chambers placed inside at either end of a 90 x 44 x 38 cm (150 L) glass aquarium (Fig. 1). Each of the aquaria contained 2 cm of coral sand substrate and one thermometer, air stone, and electric heater. The stimulus aquaria were chemically isolated from each other and from the focal fish. A 10 cm area (corresponding to 2 body lengths of a typical focal fish) in front of each stimulus chamber was designated as a choice zone.

2.1.3. Treatment

We gave each focal fish an intraperitoneal injection of 25 μl/g body mass with a 31-gauge 0.3 ml insulin syringe. To prepare the treatments, we dissolved isotocin ([Ser^4, Ile^8]-oxytocin; Bachem, Torrance CA, USA) in 0.9% saline and used this to create three different doses: 1) a low dose of 0.1 ug/g body mass, 2) an intermediate dose of 1 ug/g body mass and 3) a high dose of 5 ug/g body mass. The intermediate dose corresponded to a dose that has previously been shown to have effects on social behavior in this species [63] and is similar to nonapeptide doses that have been used in other fish species [19,45,61,71,72] The fourth, control, treatment consisted of an injection of the vehicle only (0.9% saline). All fish recovered immediately from the injections and handling, exhibiting no obvious signs of distress.

2.1.4. Procedure

We tested a total of 80 focal fish (40 of each sex) in this experiment. We selected focal fish haphazardly from the communal housing aquarium and sexed them by examination of their external genitalia. We recorded the standard length (to the nearest 0.1 mm) and body mass (to the nearest 0.01 grams) of each focal fish. We then haphazardly selected four stimulus individuals of the same-sex as the focal fish from the stimulus-housing aquarium. The four stimulus fish were separated into a group of three fish and a single fish that were placed into one of the two stimulus chambers randomly based on a coin flip. Past research on other fish species and with *N. pulcher* has shown that fish possess the numerical abilities make this type of discrimination [1,12,64]. Same-sex fish were used as stimuli so that association preference decisions represented social partner choice rather than mate choice [16,64]. All of the stimulus fish that we used were larger than the focal fish. Because *N. pulcher* show a rigid size-based dominance hierarchy [13,65,85] the focal fish would therefore assume the lowest dominance rank while associating with either the lone individual or the group of 3 fish [64]. Stimulus fish were unrelated to the focal fish.

All trials were conducted in the afternoon between 12:00 and 17:00 to control for the possibility of diurnal effects on sociality. Focal fish were injected with one of the 3 IT treatments or with the saline control by an experimenter blind to the treatment condition. Following the injection, focal fish were immediately introduced into the middle section of the social choice apparatus (Fig. 1) and allowed to acclimate to the novel aquarium for 5 min. After this 5 min, the entire aquarium was filmed from the front for 10 min. The fish were then left undisturbed and not filmed for 30 min. During this 30 min period, the focal fish was free to inspect each of the stimulus groups and swim freely around the central compartment of the social choice apparatus. Finally, the focal fish was filmed again for 10 min to ascertain the stability of its grouping preference over time and for the time course of the effect of the IT manipulation. The time course of the effect of exogenous IT in fish is not well known. However, mammalian oxytocin has a short half-life in the blood (on the order of minutes; [53]). Previous studies on *N. pulcher* found that behavioral effects of IT manipulations lasted for at least half an hour [63], that short-term grouping preferences reflect ultimate decisions to join a group [36] and that grouping preferences are consistent over time [64]. Focal fish were used only once. Stimulus fish were used only once per day but were replaced into their housing aquarium and were reused in different combinations across days.

2.1.5. Behavioral scoring

A trained observer, blind to both the sex and the treatment group of the focal fish, scored all of the video recordings. During each 10 min observation period, we recorded the time that the focal fish spent with the majority of its body including its head within each of the 10 cm preference zones adjacent to each stimulus chamber. We also recorded the number of times the focal fish touched its head against the wall, swam up and down along the glass separating one the stimulus chambers from the central compartment, or displayed to the stimulus fish. We considered these interactions to be attempts by the focal fish to access the stimulus fish, and therefore indicative of motivation to interact with those individuals [37,43]. Previous research in fish and birds has shown that it is worthwhile to consider association time and interactions separately and that interaction rate may be a more sensitive measure of motivation to affiliate than association time [37,43]. For the purposes of analysis and data presentation, we subtracted the time spent in the choice zone of the single stimulus individual from the time spent in the choice zone of the group of three stimulus individuals to produce a single grouping preference score for each focal fish. Likewise, we subtracted the number of interactions initiated across the barrier with the single stimulus individual from the number of attempted interactions with the group of three stimulus fish to produce a single social interaction score for each focal fish in this experiment. We also examined whether sex or our IT manipulation had an effect on the tendency to associate and/or interact with conspecifics in general regardless of group size. We summed both the time spent in association with either of the two stimulus groups and the number of interactions with either of the stimulus groups to produce overall association time and overall interaction rate scores for each fish during each observation period.

2.1.6. Statistical analyses

We used a two-way analysis of variance (ANOVA) model with IT treatment, sex and their interaction as independent factors for each observation for each of our two behavioral measures of sociality (time and interaction rate) as well as our two measures of general social motivation (total association time and total interactions with either group). When we found a main effect of IT treatment, we conducted Fisher's LSD post-hoc tests to determine which treatment groups differed. We tested the residuals from our statistical models for adherence to parametric assumptions and found no violations. Data are represented in figures as mean ± S.E.M. All analyses were conducted using SPSS 20 (IBM, Chicago, IL, USA) for Macintosh.

2.2. Results

2.2.1. Association time

During the first 10 min observation period, there was a significant effect of both IT treatment (2-way ANOVA; $F_{3,72} = 2.75$, $p = 0.049$) and sex ($F_{1,72} = 13.262$, $p = 0.001$) on the preference to associate with the large group versus the small group (Fig. 2a). There was no interaction between IT treatment and sex on this preference

($F_{3,72} = 0.09$, $p = 0.97$). As was found in a previous study (Reddon et al. 2011a), males had a stronger preference for associating with the large group than did females. In both sexes, the fish that received the low (Fisher's LSD; $p = 0.025$) or the high dose ($p = 0.036$) of IT showed a reduced preference for associating with the large group compared to the fish that received the saline control injection (Fig. 2a). The dose also influenced the total time focal fish spent in any association zone ($F_{3,72} = 4.12$, $p = 0.009$). Again fish that received the high dose or the low dose spent less time in association with conspecifics (low: $p = 0.013$; high: $p = 0.003$). Males tended to spend more time than females associating with conspecifics in either group, however this trend did not reach significance (2-way ANOVA, effect of sex: $F_{1,72} = 3.51$, $p = 0.065$). There was no significant interaction between sex and treatment on the tendency to associate with either of the stimulus groups ($F_{3,72} = 0.44$, $p = 0.73$).

During the second observation period, there were no effects of IT treatment, sex or an IT treatment by sex interaction on the preference for the large versus the small group (2-way ANOVAs; all F's < 0.72, all p's > 0.54; Fig. 2a). Across treatments, both males and females showed a preference to associate with the larger group (Fig. 2a). During this second observation period, there was no significant effect of sex, IT treatment or a treatment by sex interaction on the tendency to associate with conspecifics in general (all F's < 1.56, all p's > 0.21).

2.2. Social interactions

During the first observation period, we found a significant effect of sex (2-way ANOVA; $F_{1,72} = 7.60$, $p = 0.007$; Fig. 2b) on the number of interactions with the larger group compared to the small group. On average, males interacted more with the large group than did females. There was no significant effect of the IT treatment on the preference to interact with the large group compared to the small group ($F_{3,72}$ = 2.09, p = 0.108; Fig. 2b), nor was there any statistically significant interaction between dose and sex ($F_{3,72}$ = 0.08, p = 0.97; Fig. 2b). The data did, however, mirror the pattern we observed for association time, whereby fish that received either the low or the high dose of isotocin tended to interact less with the larger group when compared to the small group (Fig. 2b).

During the second observation period we did not find any significant effect of sex, IT treatment or the interaction between IT treatment and sex (2-way ANOVAs; all $F < 1.46$, all $p > 0.23$; Fig. 2b) on the tendency to interact with the large group compared to the small one. Both males and females attempted to interact with the group of three stimulus fish more often than the lone stimulus animal (Fig. 2b) but the IT treatment did not appear to affect this preference.

During both observation periods, there was no significant effect of IT treatment, sex or a treatment by sex interaction on the tendency for the focal fish to interact with any of the stimulus fish in general (all F's < 0.14, all p's > 0.13).

3. Experiment 2 – selective isotocin receptor antagonist:

3.1. Methods

3.1.1. Study animals

The study animals used in this second experiment were drawn from the same population as in experiment 1 and were housed in the same way. However, no focal fish from experiment 1 was reused as a focal fish in experiment 2. We tested a total of 40 focal fish in the second experiment, 20 of each sex. Some of the stimulus fish from experiment 1 may have been reused in experiment 2 in novel combinations.

3.1.2. Testing apparatus

The testing apparatus was identical to that used in experiment 1 (Fig. 1).

3.1.3. Treatment

We acquired a selective oxytocin/isotocin receptor antagonist (ITA; desGly-NH^2-d(CH^2)5[D-Tyr_2,Thr_4]OVT) as a generous gift from Professor M. Manning. This antagonist has been successfully used to alter behavior in fish [7,55]. We dissolved the ITA into 0.9% saline and produced three different treatment doses of ITA in addition to a saline control. We based our intermediate dose (0.5 µg/g body mass) on the antagonist dose that has been used to alter parental care behavior in convict cichlid fish [55] and on the dose of a similar antagonist that has been successfully used to alter sociality in birds [22]. We also prepared treatment doses that were half (0.25 µg/g body mass; low dose) and double the previously used dose (1 µg/g body mass; high dose) to determine if the response to this antagonist is dose-dependent. As in experiment 1, focal fish received an intraperitoneal injection of one of the 4 treatments from an experimenter who was blind to the treatment group, immediately prior to being introduced into the social choice apparatus.

3.1.4. Procedure

The procedure of experiment 2 was similar to that use in experiment 1 except that the focal fish was recorded during four 5 min blocks every 10-15 min starting 5 min after injection and introduction into the social choice apparatus (observation 1 = 5–10 min post-injection; observation 2 = 20–25 min post-injection; observation 3 = 35–40 min post-injection; observation 4 = 55–60 min post-injection). We implemented this minor change in procedure in order to get finer scale data on the time course of the effects of the antagonist, which has only been used in fish in two previous published reports, neither of which includes detailed time course data [7,55]. We do not know the effective time course of ITA, however previous research has shown effects lasting at least an hour in another species of cichlid [55]. The focal fish were free to swim about the social choice apparatus observing and interacting with both stimulus groups prior to the first observation period and between each successive observation period.

3.1.5. Behavioral scoring

Behavioral scoring was identical to experiment 1.

3.1.6. Statistical analysis

As with experiment 1, we used a two-way ANOVA model with ITA treatment, sex and their interaction as independent factors for each observation for each of our two behavioral measures of sociality. Where we found a main effect of ITA treatment, we conducted Fisher's LSD post-hoc tests to determine which treatment groups differed. We tested the residuals from our statistical models for adherence to parametric assumptions and log-transformed and retested the residuals when violations were detected. Log transformation was successful in normalizing our residuals in all cases. Data are represented in figures as mean ± S.E.M. of the untransformed data. All analyses were conducted using SPSS 20 (IBM, Chicago, IL, USA) for Macintosh.

3.2. Results

3.2.1. Association time

During the first observation period (5-10 min post-injection) there was no significant main effect of either ITA treatment (2-way ANOVA; $F_{3,32} = 0.90$, $p = 0.45$) or sex ($F_{1,32} = 0.02$, $p = 0.90$) on time the focal fish spent with the large group compared to the small group (Fig. 3a). However, there was a marginally non-significant interaction between ITA treatment and sex ($F_{3,32} = 2.49$, $p = 0.078$), whereby males but not females that were given the high dose (1μg/g body mass) of ITA tended to prefer the larger group over the small group (Fig. 3a).

During the first observation there was a sex by ITA treatment interaction on the time spent associating with either of the stimulus groups ($F_{3,32} = 3.33$, $p = 0.032$). However, when we broke this interaction down by sex, the treatment effect did not reach significance for either males ($F_{1,16} = 1.91$, $p = 0.17$) or females ($F_{1,16} = 2.64$, $p = 0.09$) separately.

During the second, third and fourth observation periods, we did not find any effect of sex, dose nor a sex by ITA treatment interaction on the preference for *N.*

pulcher to associate with the group of three fish compared to one fish (2-way ANOVAs; all F's < 1.9, all p's > 0.17; Fig. 3a). Likewise, during the latter 3 observation periods, there was no significant effect of ITA treatment, sex or a sex by treatment interaction on the time spent associating with either of the stimulus groups (all F's < 2.82, all p's > 0.06).

3.2.2. Social interactions

During the first observation period (5 – 10 min post-injection) we found a significant main effect of the ITA treatment (2-way ANOVA; $F_{3,32} = 3.86$, p = 0.018; Fig. 3b) on the number of attempts to interact with the large group versus the small group whereby fish given the highest dose of ITA showed the most interactions with the large group compared to the small group. However, there was also a significant sex by treatment interaction ($F_{3,32} = 3.39$, p = 0.03). In order to unpack this interaction, we re-ran the analysis separately for males and females. Male *N. pulcher* showed a significant ITA treatment effect (2-way ANOVA; $F_{1,16} = 6.17$, p = 0.005; Fig. 3b). Specifically, males that received the highest dose of ITA (1µg/g body mass) showed a pronounced tendency to interact with the group of three stimulus fish more than the lone stimulus individual, interacting significantly more with the large group than males given a control injection of saline (Fisher's LSD; p = 0.016), the low ITA dose (p = 0.001), or the intermediate ITA dose (p = 0.018). Females, by contrast, did not show any significant effect of treatment during the first observation period (2-way ANOVA; $F_{1,16} = 0.91$, p = 0.46; Fig. 3b). During the first observation there was a sex by ITA treatment interaction on the total number of interactions with either of the stimulus groups ($F_{3,32} = 302$, $p = 0.044$). However, when we broke this interaction down by sex, the treatment effect did not reach significance for either males ($F_{1,16} = 2.06$, $p = 0.15$) or females ($F_{1,16} = 0.98$, $p = 0.43$).

We did not find any effect of sex, ITA treatment nor a sex by ITA treatment interaction on the preference to interact with the large group compared to the lone stimulus individual during any of the 3 latter observation periods (2-way ANOVAs; all $F < 2.03$, all $p > 0.13$; Fig. 3b).

During the second observation period, there was a significant ITA dose by sex interaction on the total number of interactions across stimulus groups ($F_{3,32} = 3.36$, $p = 0.031$). However, when we separated this analysis by sex to decompose this interaction, we did not find a significant treatment effect in either sex (males: $F_{1,16} = 1.83$, $p = 0.18$; females: $F_{1,16} = 2.21$, $p = 0.13$). During the third and fourth observation periods there was no effect of sex, ITA treatment or an ITA treatment by sex interaction on the total number of interactions with any of the stimulus fish across groups (all F's < 2.62, all p's > 0.07).

4. Discussion

We examined the role of the oxytocic hormone system in regulating sociality in the cooperatively breeding cichlid fish *Neolamprologus pulcher*. In our first experiment, we injected male and female *N. pulcher* with one of three different doses of exogenous IT or a saline control and examined group joining preferences. Contrary to our predictions, we found that an intermediate dose of IT did not increase the preference to associate with more individuals. Also the high (and low) dose of IT reduced rather than increased the large group preference in *N. pulcher.* Furthermore, high (and low) doses of IT reduced the tendency for *N. pulcher* to associate with conspecifics of either group size, suggesting this treatment had a general anti-social effect. In our second experiment, we administered one of three doses of an isotocin receptor antagonist or a saline control and again contrary to our predictions, we found that a high dose of ITA increased rather than decreased sociality in males, whereas females were unaffected by any dose. The effect of our treatments appeared to wear off rapidly, and in both experiments we saw treatment differences in behavior only during the first observation period within the first 10-15 minutes following the injection.

Collectively, we detected relatively weak evidence that IT is an important proximate regulator of sociality in *N. pulcher*. While we did document differences in behavior at certain doses of both IT and ITA, these differences tended to be fairly muted, and were inconsistent with our predictions and transient in time course. The behavioral effects we detected with in both experiments were for the most part confined to the first observation period. Our study joins an increasing number of reports that challenge the naïve prediction that the oxytocic hormone system has a general prosocial effect across species and contexts (see reviews: [8,23]). A more complex picture of the role of oxytocin and its homologues in regulating social behavior is emerging, and it is clear that blanket predictions across taxa are currently not possible [23].

N. pulcher are an obligate grouping species and are never found alone or in breeding pairs without subordinates [4,80,84]. Perhaps because social behavior is so ingrained into the life history of this species, the sociality response may be highly canalized and supported by multiple interacting physiological systems [28,54,57,58]. A previous study did find behavioral effects of exogenous IT on more subtle aspects of *N. pulcher* social behavior [63], so it is possible that IT predominantly regulates more fine-scale and context-specific aspects of *N. pulcher* behavior, while sociality is too fundamental to the behavioral ecology of *N. pulcher* to observe a strong effect of acute IT manipulations. If sociality is so firmly entrenched into the neurobiology and physiology of *N. pulcher*, then acute augmentation or disruption of a single socially relevant system (in this case, isotocin) may be insufficient to produce dramatic differences in grouping behavior. Further experimentation with other highly social species, species that are facultatively social, and species that are relatively non-social will help to clarify this issue.

Another possible explanation for our results stems from the fact that our manipulations were delivered peripherally, with the assumption that both IT and ITA pass sufficiently into the brain to have a centrally-mediated effect on behavior. The majority of the vast literature on exogenous oxytocin effects on human behavior is based on the premise that peripherally administered oxytocin is reaching the brain in pharmacological quantities (see [50] for a review and [8] for a critique), and our study joins a growing literature that has reported behavioral effects from peripherally administered nonapeptides and blockers (e.g., [7,19,22,45,51,52,61,63,71,72]). Thus, there is good evidence that these peripheral treatments can generate behavioral effects. However, it is possible that one or both of our treatments did not pass into the brain at a sufficient dose to have an effect on central receptors and instead exerted its effects through an indirect pathway involving peripheral receptors. The blood-brain barrier generally has low permeability to nonapeptides [8,53,66,67]. Interestingly, fish blood-brain barriers appear to have greater permeability to neuropeptides than in birds or mammals [6,59]. There is also evidence that peripheral and central actions of nonapeptides are tightly integrated [26,68-70] and activation of peripheral receptors may therefore result in downstream effects on behavior mediated ultimately by central nonapeptide systems. The additional step added by the peripheral pathway may explain some of our unexpected results; for example, high doses of IT in the periphery could lead to a compensatory downregulation of endogenous central release of IT, and therefore lead to paradoxically opposite responses than those predicted *a priori*. There is a growing appreciation that peripheral receptors may play an important role in regulating social behavior [8,23,38,60]. The mounting evidence demonstrating behavioral effects from peripheral nonapeptide manipulations, sometimes with unexpected results, suggests that further research on peripheral nonapeptide effects is needed.

In summary, we explored the effects of manipulations to the oxytocic system on sociality in a highly social cichlid fish, *N. pulcher*. We found some support for the hypothesis that the oxytocic hormone system is a regulator of sociality, namely, that both exogenous isotocin and a specific isotocin receptor antagonist altered behavior in a test for sociality. However, the effects we observed tended to be weak, transient and not in the predicted direction. This study joins a small number of published reports explicitly investigating the role of the oxytocic hormone system in regulating sociality and a small number of studies looking at the effects of experimental isotocin manipulations on social behavior in fish. Our results highlight the need for additional research on a greater diversity of taxa exhibiting a variety of social systems. Without these additional data, it is not currently possible to make strong directional predictions about the role that the oxytocic system plays in regulating sociality and social behavior in general across the diversity of animal life.

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Figure captions

Fig. 1. A schematic representation of the social choice apparatus as viewed from the front of the aquarium. The dashed lines delineate the preference zone for each stimulus chamber (10 cm).

Fig. 2. (A) The average time fish spent associating with the large group, minus the time spent associating with the small group during each of two observation periods when individuals were treated with one of three experimental doses of isotocin or a vehicle only control. We found significant effects ($p < 0.05$) of isotocin dose and sex during the first observation, whereby the high and low doses reduced sociality, and males showed greater sociality than females. (B) The average number of interactions (through the glass barrier) with the large group, minus the number of such interactions with the small group during each of two observation periods. Again, we found a significant effect ($p < 0.05$) of sex during the first observation, whereby males showed greater sociality than females.

Fig. 3. (A) The average time fish spent associating with the large group, minus the time spent associating with the small group during each of four observation periods following treatment with one of three experimental doses of a specific isotocin receptor antagonist or a vehicle only control. We did not find any significant effect of sex or treatment during any of the observation periods. (B) The mean number of interactions with the large group, minus the number of interactions with the small group during each of four observations following treatment with one of three experimental doses of a selective isotocin receptor antagonist or a vehicle only control. We found a significant effect ($p < 0.05$) of treatment on sociality in

males but not females during the first observation period, whereby males that received the high dose showed a greater sociality response.

Figure 1.

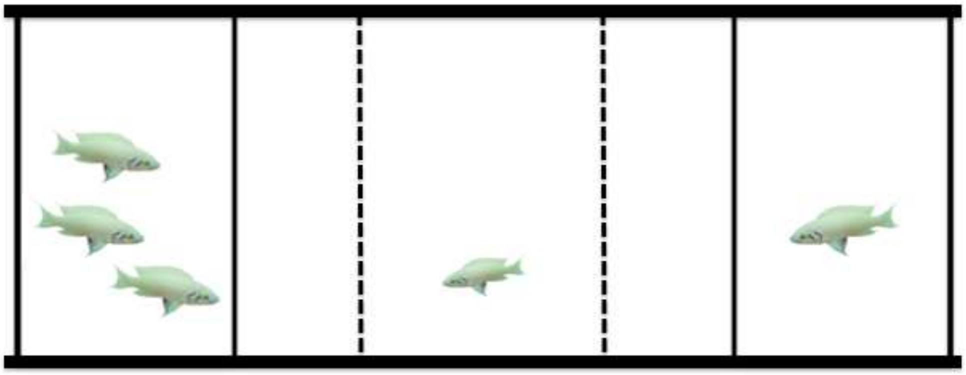


Figure 2.

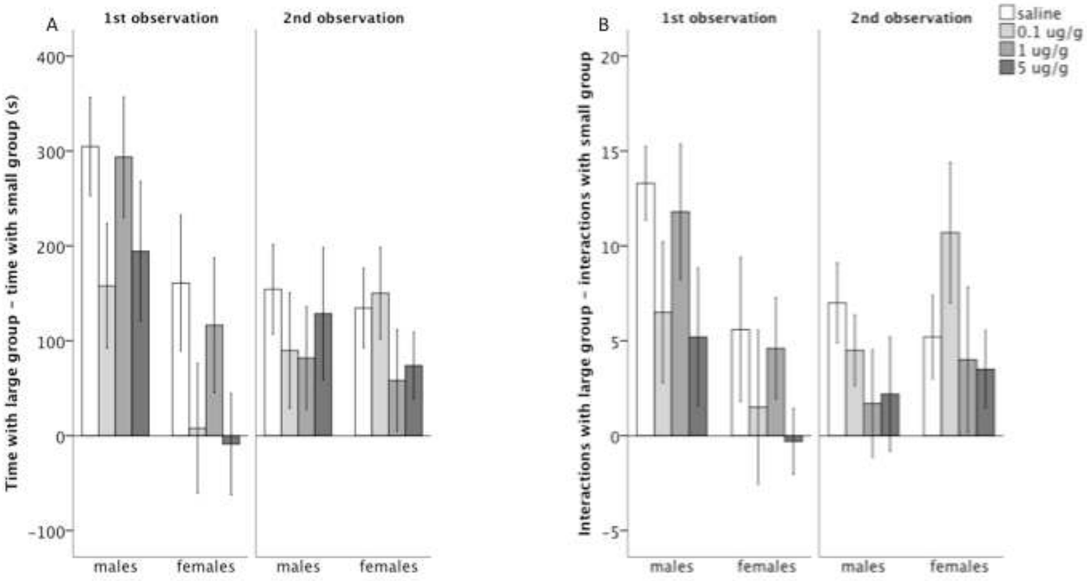
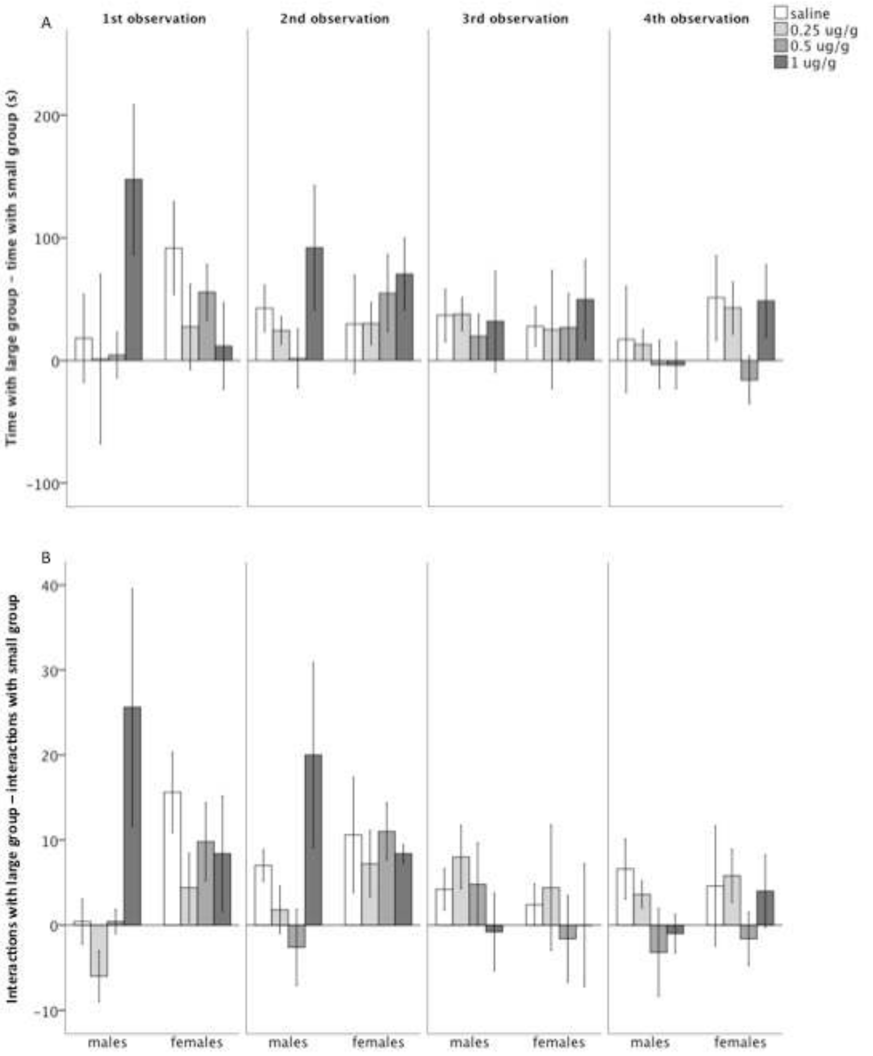

A
1st observation
2nd observation
Time with large group - time with small group (s)
400
300
200
100
0
-100
males
females
males
females
B
1st observation
2nd observation
Interactions with large group - interactions with small group
20
15
10
5
0
-5
males
females
males
females
saline
0.1 ug/g
1 ug/g
5 ug/g

Figure 3.


A
1st observation
2nd observation
3rd observation
4th observation
saline
0.25 ug/g
0.5 ug/g
1 ug/g
Time with large group – time with small group (s)
200
100
0
-100
B
Interactions with large group – interactions with small group
40
30
20
10
0
-10
males
females
males
females
males
females
males
females

Chapter 7: Network structure is related to social conflict in a cooperatively breeding fish

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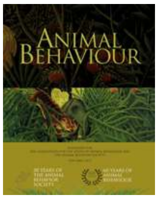

Network structure is related to social conflict in a cooperatively breeding fish

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within-group interaction

The nature of individual social interactions can have a profound influence on group structure and function. Here, we use social network analysis to examine patterns of dominance interactions and spatial associations in 14 captive social groups of the cooperatively breeding cichlid, *Neolamprologus pulcher*. In this cichlid, social groups are composed of a dominant breeding pair and 1–20 nonbreeding subordinate helpers that form size-based queues for breeding positions. In the current study, we performed the first quantitative analysis of *N. pulcher* dominance hierarchies. We found that dominance hierarchies of *N. pulcher* were highly linear and that interactions within dyads were directionally consistent. We also found that dominance interactions were not equally distributed across the network, but instead occurred most frequently at the top of the social hierarchy. Contrary to our predictions, neither body size asymmetry nor sex predicted the observed dominance interactions and patterns of associations. However, breeders were more connected than helpers within the networks, perhaps due to their policing role. This study is one of a small handful to conduct network analysis on replicate social groups, and thus is one of few studies able to make general conclusions on the social structure of its focal species. The patterns uncovered suggest that conflict over breeding position inheritance has a strong impact on relationships among group members in *N. pulcher*.

Many different species live in groups, and the evolution and maintenance of such social structure strongly depends on the nature of the interactions among individuals. Within social groups, behavioural interactions typically occur nonrandomly among group members (Dugatkin & Sih 1995; Krause et al. 2007). Individuals often preferentially interact with particular social partners because variation in individual attributes (e.g. age, resource-holding potential, sex, personality) causes specific social interactions to be costly or beneficial (Krause 1994; Lusseau & Newman 2004; Pike et al. 2008; Schürch et al. 2010). However, even if all group members are functionally similar, nonrandom interactions may be inherently beneficial. For example, in many species, individuals preferentially group with familiar individuals, because associating with known partners leads to lower aggression and higher foraging success (reviewed in: Griffiths 2003; Ward & Hart 2003). Generally, variation in individual social interactions can influence the structure and function of social groups (Flack et al. 2006), which will in turn influence individual fitness (e.g. Ryder et al. 2008; Silk et al. 2009; Oh & Badyaev 2010). More specifically, patterns of social interactions dictate many aspects of social living, such as the allocation of reproduction (e.g. Herrera & Macdonald 1992), the evolution of cooperation (e.g. Ohtsuki et al. 2006; Voelkl & Kasper 2009), and the transmission of information or disease (Krause et al. 2007; Wey et al. 2008; Godfrey et al. 2009).

Social network analysis provides a quantitative framework for analysing patterns of interactions among individuals (Croft et al. 2004). In its basic form, a social network is composed of individuals (represented by nodes) that are connected by their interactions (represented by edges; Whitehead 2008). In addition to these relational data, attributes of individuals can also be laid onto the network (Croft et al. 2008). Therefore, the network approach allows for analysis of behaviour in the context of an individual's social environment, facilitates exploration of the emergence of behavioural phenotypes at the group or population level (Croft et al. 2008), and is a promising tool for understanding the link between individual traits and group- or population-level phenomena.

While social network analysis has been increasingly employed in behavioural biology (reviewed in: Krause et al. 2007; Wey et al. 2008; Sih et al. 2009), few studies have analysed the network structure of multiple independent social groups of a given species. Indeed, Croft et al. (2008, page 146) noted that such replication is 'conspicuously absent in many network studies'. This trend is beginning to change (e.g. see recent studies by Croft et al. 2005;

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Thomas et al. 2008; McCowan et al. 2008; Madden et al. 2009; Schürch et al. 2010). However, more studies that compare network structure among replicate social groups are clearly warranted if we wish to reach general conclusions about the causes and consequences of the structure of animal societies. For animals that readily perform natural behaviour in captivity, analysing the network structure of captive groups provides a feasible means of gathering data on multiple replicate groups under controlled conditions. While studying the social networks of captive animals may have some drawbacks (e.g. these captive social groups may not precisely mimic the composition of natural groups), there are also advantages in that researchers can more easily manipulate or control factors predicted to affect network structure, and can therefore reach robust conclusions.

Here, we investigate behavioural interactions within social groups in the cooperatively breeding cichlid, *Neolamprologus pulcher*. This species is endemic to Lake Tanganyika, Eastern Africa, and forms permanent socials groups composed of a single dominant breeding pair, and 1–20 male and female subordinate helpers (Taborsky 1984, 1985; Balshine et al. 2001; Heg et al. 2005). The breeding male is always the largest individual, and the breeding female is typically the second-largest individual (Wong & Balshine 2011a), while the nonreproductive helpers form a size-based hierarchy thought to reflect two sex-specific queues for breeding status (Balshine-Earn et al. 1998; Werner et al. 2003; Heg et al. 2004; Hamilton et al. 2005; Fitzpatrick et al. 2008; Mitchell et al. 2009).

To better understand intragroup dynamics in *N. pulcher*, we explored how social conflict influences the structure of social networks. Social conflict may be manifested in aggressive, submissive and/or avoidance behaviours (Balshine-Earn et al. 1998; Aureli & de Waal 2000; Werner et al. 2003; Hamilton et al. 2005; Reddon et al. 2012). Thus, we test five predictions related to social conflict and the structure of dominance and association networks.

(1) There have been widespread claims that *N. pulcher* groups form linear dominance hierarchies (Taborsky & Limberger 1981; Taborsky 1984, 1985; Wong & Balshine 2011a, b; Reddon et al. 2011a, b). However, we are unaware of any specific tests of hierarchy structure in this species. Based on these prior assertions, we predicted that *N. pulcher* dominance hierarchies would be linear, and we performed the first test of this prediction using a quantitative analysis of hierarchy linearity and asymmetry (i.e. directional consistency).

(2) In size-structured groups, conflict is predicted to be highest between individuals of similar size (Enquist et al. 1987; Jennions & Blackwell 1996; Hamilton et al. 2005), either because relative fighting ability is uncertain (Enquist & Leimar 1983) or because subordinates should challenge dominants more frequently if the difference in fighting ability is small (Cant & Johnstone 2000). Therefore, we tested the prediction that dyads with low size asymmetry would be involved in more frequent dominance interactions and would have fewer associations with one another.

(3) Only same-sex individuals are expected to compete for breeding positions. Therefore, we tested the prediction that dominance interactions would occur more frequently and associations would occur less often between same-sex group members.

(4) As the value of a social position rises exponentially with increasing rank, dominance interactions are theoretically expected to most frequently occur towards the top of the dominance hierarchy (Cant et al. 2006). Therefore, we tested this prediction by quantifying the rate of dominance interactions throughout the hierarchy, and explored whether high-ranking group members were involved in more dominance interactions than low-ranking members.

(5) Female *N. pulcher* queue for breeding positions within their natal groups while males more commonly disperse to another group prior to breeding (Stiver et al. 2004, 2006, 2007). We therefore predicted that females would place more value on establishing relationships with group members, and would consequently be more connected within the networks than males. Similarly, we predicted that breeders would be more connected than helpers, because they have a greater interest in the structure of their current group than do the subordinate helpers.

METHODS

Study Animals

Neolamprologus pulcher used in this study were adults from a breeding colony held at McMaster University, Hamilton, ON, Canada. The fish were descendants of breeding pairs caught in Lake Tanganyika, Zambia, and were housed in social groups consisting of a male and female dominant breeding pair with either three or four subordinate helpers of mixed sexes (mean group size ± SE = 5.8 ± 0.1). This group size and composition is consistent with the structure and size range of wild *N. pulcher* social groups found in Lake Tanganyika (Balshine et al. 2001). The relative size of male and female helpers as well as the ratio of male to female helpers varied among the social groups, but there was always at least one male and one female helper, and there were always both high-ranking and low-ranking helpers within the groups. Each social group inhabited a 189-litre (92 × 41 × 50 cm) freshwater aquarium outfitted with a heater, thermometer, two filters, about 3 cm of crushed coral sand substrate, and two inverted terracotta flowerpot halves for use as shelters and spawning sites. Social groups were formed approximately 1 month prior to the start of behavioural observations (see below) and each social group had successfully reproduced at least once prior to the start of the study. Fish were fed ad libitum 6 days per week with commercial cichlid flake food.

Study Protocol

In total, 14 social groups were used in this experiment. All fish were captured, weighed, measured, sexed by external examination of their genital papillae, and given a unique fin clip 48 h prior to the first observation, so that each fish could be individually identified. Groups were observed for 15 min twice a week for 2 consecutive weeks, for a total of four observation periods and 60 min of observation per group. During each observation period, a pair of observers simultaneously watched each group from a distance of approximately 1.5 m. Fish were given 5 min to acclimate to the presence of observers prior to the onset of recording, and the fish did not appear disturbed by the presence of human observers. One observer scored associations among individuals, recording the individuals within a single body length of each other at the beginning of each minute. A single body length was chosen since this is the spatial distance that social interactions occur in *N. pulcher* and is a widely used spatial metric in fish behavioural studies. The second observer continuously recorded all dominance-related behaviours among all group members, based on a recent ethogram for this species (Sopinka et al. 2009). Specifically, this observer recorded all aggressive displays and behaviours (aggressive postures, puffed throats, head shakes, rams, chases and bites) and all submissive behaviours (submissive postures and submissive displays) that were both produced and received by each fish in the group.

Social Networks

Using this data, we built two social networks, one was based on dominance interactions, and the other was based on associations, for the individuals in each social group. In each network, the weight of connecting edges was determined by summing the number of interactions or associations for each dyad across the four observation periods (see above). Note that *N. pulcher* social networks were stable over the study time period, as networks built from the first two observation periods were highly positively correlated to networks built from the second two observation periods (Mantel tests followed by Fisher's omnibus test (Louv & Littell 1986; Haccou & Meelis 1992): dominance networks: mean $r_S = 0.55$, $f_{28} = 100.4$, $P < 0.0001$; association networks: mean $r_S = 0.46$, $f_{28} = 75.9$, $P < 0.0001$). The network of dominance interactions was created by combining the sociomatrix of aggressive interactions (a matrix in which columns and rows represent individuals, thus each cell in the matrix represents a dyad) with the transposed sociomatrix of submissive interactions (i.e. the actor and receiver are reversed), for each group. Dominance networks had no maximum edge weight, and could be either directed (when exploring dominance relationships as per prediction 1) or undirected (when we used the total number of dominance interactions as a measure of social conflict; as per predictions 2–4), depending on the analysis employed. The association network was undirected, and each dyad had a maximum edge weight of 60 (i.e. if the dyad was associated during every scan across the four observation periods). Four of the 56 subordinate helpers died during the study period and these individuals were removed from all networks. There were no self-loops in any networks (i.e. the diagonal in all sociomatrices was set to 0) and this feature was conserved during all randomization tests.

Network Analysis

Network analysis was performed in R version 2.14.1 (R Development Core Team 2012) and UCINET version 6 (Borgatti et al. 2006). During randomization tests, we held the total number of interactions or associations constant within each social group. When appropriate, we used Fisher's omnibus test (Louv & Littell 1986; Haccou & Meelis 1992) to combine *P* values obtained from each social group into a single value. We determined the direction of the strongest relationship among groups, and subtracted the contribution of groups with the opposite relationship from the combined test statistic, and the resultant overall *P* value (see also Croft et al. 2006). Networks in Figures 1a, b were created using the 'igraph' package in R (Csárdi & Nepusz 2006).

Dominance hierarchy structure

To test prediction 1, we examined the structure of dominance hierarchies using the triangle transitivity method recently developed by Shizuka & McDonald (2012). This measure of dominance hierarchy structure is equivalent to linearity (sensu Landau 1951; de Vries 1995) when all pairwise dominance relationships are known. However, it is advantageous in that it does not become biased when pairs of individuals have not interacted (see Klass & Cords 2011), and/or when group size varies (Shizuka & McDonald 2012). The directed dominance matrix was reduced to a binary dominant/subordinate matrix (1 = dominant, 0 = subordinate) based on which individual in each dyad had a larger value in the dominance matrix (i.e. which individual had 'won' more dominance interactions). If a dyad had not interacted, both members were given a 0. Next, the proportion of transitive triangle motifs (t_{tri}) was determined for this binary network, using the 'statnet' package in R (Handcock et al. 2003). The statistical significance of t_{tri} was determined by comparing the empirical value of t_{tri} with values obtained from 2000 permutations of the dominant/subordinate matrix (see Shizuka & McDonald 2012 for details). We also calculated the global asymmetry in dominance interactions across all dyads in each network. In addition to the structural organization of the dominance hierarchy (i.e. linearity or transitivity), the degree to which dominant individuals are likely to win a contest over subordinate individuals is an important characteristic of dominance relationships (van Hooff & Wensing 1987; de Vries et al. 2006; Whitehead 2008). So, for each dyad, we determined the dominant and subordinate individual (as above). Then, we divided the number of interactions in which the dominant individual behaved as such (i.e. they gave aggression or received submission) by the total number of dominance interactions in the network. The resultant statistic ranges from 0.5 to 1, and describes the global likelihood that a dominant individual would be correctly identified given an observation of a single interaction. To test whether dominance interactions among *N. pulcher* were significantly more asymmetrical than random, we performed 2000 permutations (per social group) of the raw dominance interactions. Then, we compared the dominance asymmetry score for networks built from the randomized data with our empirical values.

Network-level analysis

We tested our predictions about patterns of social conflict (predictions 2–4) using undirected dominance networks and networks of association described above. We first determined whether there was nonrandom structure in our networks (i.e. if individuals preferentially interacted or associated with particular group members). Using R, we performed 2000 permutations (per social group) of the raw dominance interaction data and compared the standard deviations in the weights of all possible edges between our observed networks and networks built from our randomized data. A high standard deviation in edge weight in our empirical networks (relative to the randomized networks) would indicate that individuals preferentially interact or associate with certain partners. Next, we used the multiple regression quadratic assignment procedure (MRQAP) in UCINET (Krackhardt 1988; Borgatti et al. 2006; see also Wey & Blumstein 2010 for a similar analysis) to regress multiple independent matrices on an observed dependent matrix. In this analysis we used three independent matrices.

(1) To test whether patterns of conflict were related to size asymmetry, we created a sociomatrix for each social group in which each element (i.e. cell) was the standard length of the larger individual divided by the standard length of smaller individual. In this size asymmetry sociomatrix, larger values indicate dyads with large differences in body size.

(2) To test whether patterns of conflict were related to sex, we created a sex-similarity sociomatrix in which a dyad was given a 1 if both individuals were the same sex, or a 0 if they were of different sex.

(3) To determine whether conflict was related to social rank, we created a sociomatrix in which each cell was the sum of the size ranks of the dyad. In *N. pulcher*, body size is highly correlated with social rank (Taborsky 1984, 1985), and size hierarchies are maintained by strategic regulation of growth (Heg et al. 2004). Thus, body size is often used to infer rank in this species (e.g. Hamilton et al. 2005). In our analysis, the value for the cell connecting the largest to the second-largest individual would be 3 (size rank 1 + size rank 2), while the value connecting the fifth-largest to the sixth-largest individual would be 11 (size rank 5 + size rank 6). Thus, a negative effect size for this matrix would indicate that dominance interactions or associations occur most often between high-ranked individuals.

We performed separate analyses for each dominance matrix for each social group, using the double Dekker semipartialling method (Dekker et al. 2007) with 2000 permutations per analysis. We performed a similar MRQAP analysis on networks of associations, except in this case we used only two independent matrices; the size asymmetry matrix and the binary sex similarity matrix described above.

Nodal measures

To investigate differences among individuals in their role in social networks (prediction 5) we compared average nodal strength and eigenvector centrality between males and females, and between breeders and helpers. Node strength measures the total weight of all edges connected to a node (Whitehead 2008), while eigenvector centrality measures how well connected an individual is by considering both the direct connections to the focal node and also the connectedness of the focal node's neighbours (Bonacich 1987; Newman 2004). For each network, we calculated an average strength and eigenvector centrality for each class of individuals (i.e. males and females, breeders and helpers). To test whether there was a significant difference between the classes, we performed 2000 permutations of the data and compared the empirical connectivity values with those calculated from networks built using the randomized data.

Ethical Note

Fish were marked with a dorsal fin clip to allow for visual identification. Fin clipping does not adversely affect behaviour (Stiver et al. 2004) and the fish recovered from this procedure immediately. The methods for animal housing, handling and experimental protocols were assessed and approved by the Animal Research Ethics Board of McMaster University (Animal Utilization Protocol Number 10-11-71) and adhere to the guidelines of the Canadian Council for Animal Care and the ABS/ASAB.

RESULTS

Neolamprologus pulcher *Groups Form Linear Dominance Hierarchies (Prediction 1)*

The dominance hierarchies were highly linear (permutation test: mean $t_{\text{tri}} = 0.80, f_{28} = 50.68, P = 0.005$). Of 133 closed triangle motifs across all 14 social groups, 128 were transitive. We also found that dominance relationships in this species were highly asymmetrical (mean dominance asymmetry $= 0.90$, $f_{28} = 188.44$, $P < 0.001$).

Conflict Is Highest between Group Members of Similar Size (Prediction 2)

Both dominance interactions and associations occurred nonrandomly throughout *N. pulcher* social groups (permutation test: $f_{28} = 189.02$, $P < 0.001$ and $f_{28} = 183.26$, $P < 0.001$, respectively; Fig. 1). However, we found no significant effect of size asymmetry on patterns of dominance interactions or associations (MRQAP: $f_{28} = 1.82$, $P > 0.99$ and $f_{28} = 7.28$, $P > 0.99$, respectively).

Conflict Is Higher between Same-sex Individuals (Prediction 3)

We found no significant effect of sex on the observed patterns of dominance interactions or associations (MRQAP: $f_{28} = 3.75$, $P > 0.99$ and $f_{28} = 9.50$, $P > 0.99$, respectively).

Conflict Is Higher Near the Top of the Social Hierarchy (Prediction 4)

Dominance interactions were significantly more likely to occur between individuals at the top of the hierarchy (MRQAP, effect of rank: $f_{28} = 65.28$, $P < 0.001$; Fig. 2).

Females Are More Connected Than Males and Breeders Are More Connected Than Helpers (Prediction 5)

In dominance networks, breeders had significantly higher strength (permutation test: $f_{28} = 139.66$, $P < 0.001$) and eigenvector centrality ($f_{28} = 133.04$, $P < 0.001$) than did helpers (prediction 5: Fig. 3). There was no difference in strength or eigenvector centrality ($f_{28} = 14.12$, $P = 0.98$ and $f_{28} = 21.74$, $P = 0.79$, respectively) between males and females (Fig. 3). In association networks, breeders had significantly higher strength ($f_{28} = 121.12$, $P < 0.001$) and eigenvector centrality ($f_{28} = 97.21$, $P < 0.001$) than helpers (prediction 5: Fig. 3). Finally, there was no difference in strength or eigenvector centrality ($f_{28} = 6.63$, $P > 0.99$ and $f_{28} = 27.21$, $P = 0.51$, respectively), between males and females in association networks (Fig. 3).

DISCUSSION

In this study, we used social network theory to explore interaction patterns within groups of the cooperatively breeding cichlid, *N. pulcher*. Consistent with our predictions, *N. pulcher* hierarchies were highly linear, with highly asymmetrical and directionally consistent interactions between dyads. While neither dominance interactions nor patterns of associations were directly related to body size asymmetry or sex, we found that dominance interactions were not equally distributed across the network, but instead occurred most frequently at the top of the social hierarchy. Finally, breeders were more connected than helpers within the networks.

We found that *N. pulcher* social groups form highly transitive (and therefore linear) dominance hierarchies with large asymmetries in dyadic dominance interactions (i.e. dominance interactions were strongly directional within dyads). Such a pattern is expected when there is large variation in resource-holding potential among individuals, and dominance ranks should therefore be predetermined by differences in individual attributes (Chase & Seitz 2011). In *N. pulcher*, as in most fishes, resource-holding potential is strongly correlated with body size (Reddon et al. 2011b) and *N. pulcher* social groups are stratified according to body size (Taborsky 1984, 1985). Therefore, there should rarely be multiple individuals with similar resource-holding potential, and as observed, dominance hierarchies should be linear and highly asymmetrical.

Within *N. pulcher* social groups, certain pairs of individuals experienced greater social conflict relative to other dyads. In agreement with our prediction, social conflict was highest towards the top of the size hierarchy. When social groups take the form of reproductive queues, there should be conflict over social status because higher-ranking individuals are more likely to inherit a breeding position. While it is beneficial for all individuals to increase their social rank, the consequences of rank change are greatest for high-ranking individuals (Cant et al. 2006). Thus, high-ranking individuals ought to invest more heavily in deterrent displays towards subordinates and in aggressive tests of dominants compared with lower-ranked individuals. In support of this idea, the aggression levels of several species of social insects (e.g. *Ropalidia marginata*, Chandrashekara & Gadagkar 1992; *Dinoponera quadriceps*, Monnin & Peeters 1999; *Polistes dominulus*, Cant et al. 2006) have been experimentally shown to be influenced by rank, rather than vice versa. Furthermore, aggression rates increase with social rank in several social vertebrates (e.g. *Equus caballus*, Araba & Crowell-Davis 1994; *Pan troglodytes*, Muller & Wrangham 2004), although such tests rarely consider confounding variables such as sex or body size. In the current study, we provide strong support for the influence of social rank on intragroup conflict in *N. pulcher*, by showing a strong correlation between dyad rank and rates of

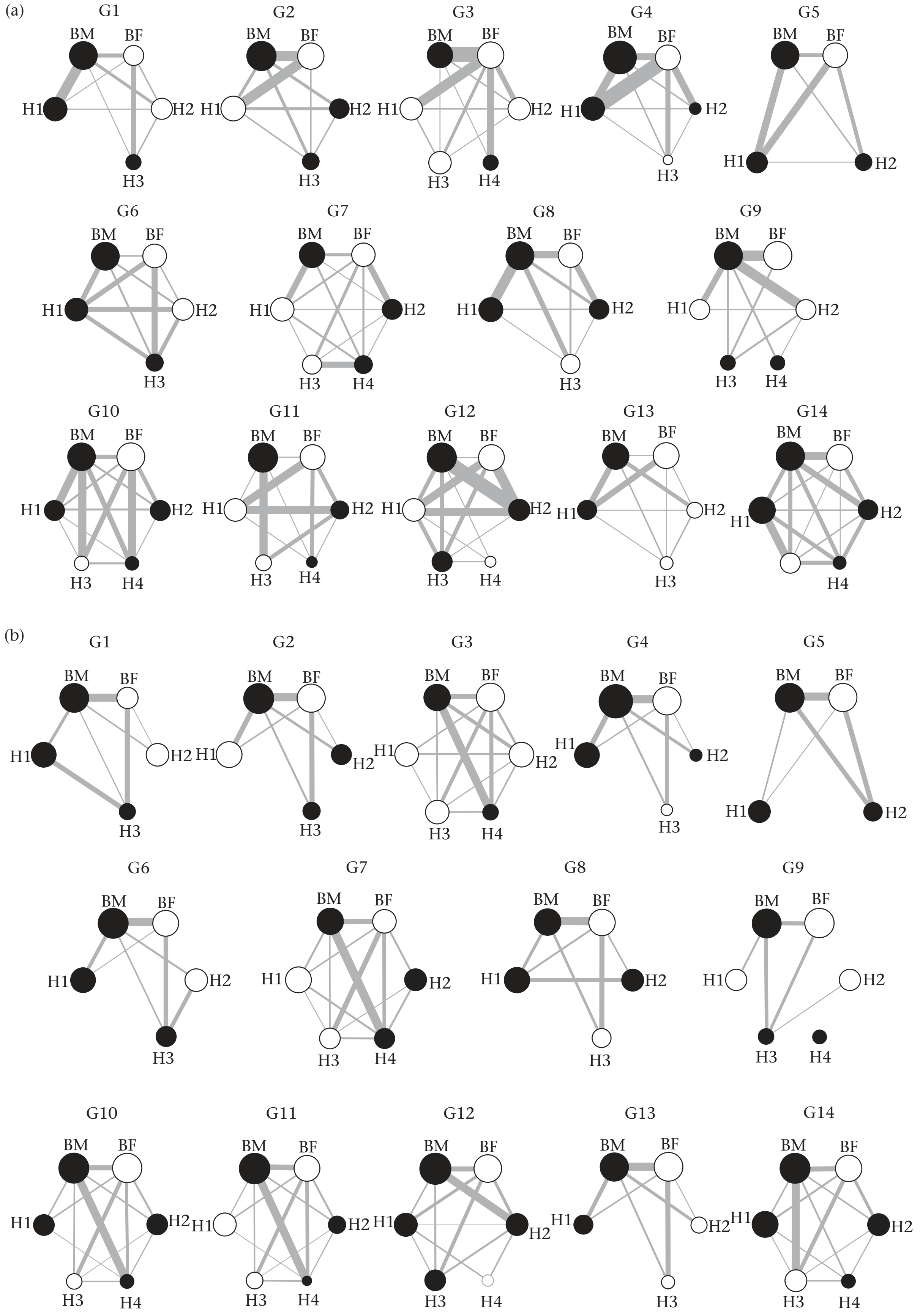

Figure 1. Networks of (a) dominance interactions and (b) associations in *Neolamprologus pulcher*. Nodes (circles) represent individual fish in each of 14 captive social groups; node labels indicate the breeding male (BM), breeding female (BF) and nonbreeding helpers in order of standard length (with H1 being the largest helper); node colour indicates sex (black = male, white = female); node size is scaled to individual body size. The thickness of connecting edges is scaled to the number of dominance interactions and the number of associations that occurred between each dyad across all observation periods and is comparable both within and among groups.

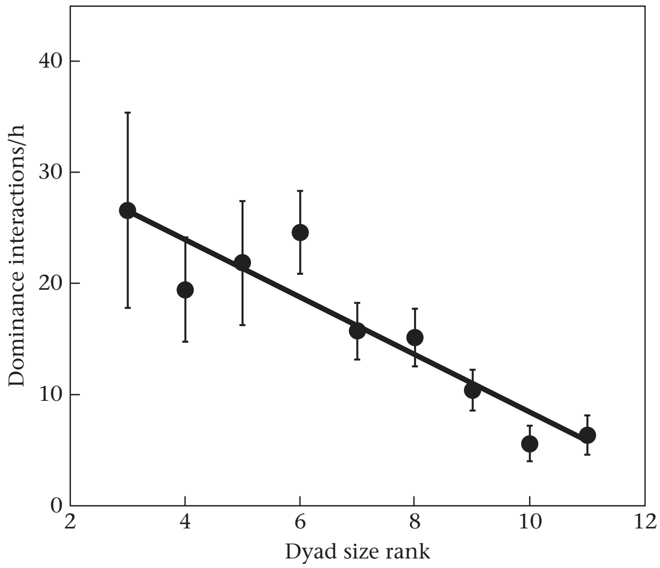

Figure 2. Mean ± SE dyadic dominance interaction rate as a function of the sum of the size rank of the members of the dyad (e.g. size rank 1 + size rank 2 = 3) in *Neolamprologus pulcher* groups. Values are pooled across all 14 social groups. The black line indicates a regression through the average values at each dyad size rank.

dominance interactions while controlling for several alternative hypotheses.

While higher-ranked individuals had more conflict with one another compared with lower-ranked individuals, neither sex nor size asymmetry were related to aggression. We had predicted increased conflict between same-sex and similarly sized individuals, since these individuals should pose the greatest threat to each other within the reproductive queue (Hamilton et al. 2005; Mitchell et al. 2009). When body size asymmetry is low, subordinates ought to challenge dominants more readily because they have a higher chance of being successful (Cant & Johnstone 2000). However, we found no relationship between sex or size asymmetry and the level of conflict. This may be because the relative value of winning a conflict is low for subordinates far down the reproductive queue. Thus, conflicts may be rare among low-ranking individuals, even when those individuals are the same sex and similar in size. As a result there may only be a weak (and in this study, nonsignificant) effect of sex and size asymmetry on the overall patterns of social conflict in a group. It is also likely that individual *N. pulcher* do not always have perfect knowledge of the sex of all other group members, especially among sexually suppressed subordinates, and thus sex-specific dominance hierarchies may simply not form. Finally, note that patterns of aggression are complex, and may be dependent on external ecological factors as well as the characteristics of the individuals involved (Reeve 2000). Furthermore, the current models used to predict patterns of aggression within social groups (i.e. reproductive skew models) consider only direct reproduction as the resource over which individuals compete, which is not appropriate for predicting aggression patterns among nonbreeding subordinates. We argue that models that incorporate future reproductive prospects (e.g. social rank) and resources not directly linked to reproduction (e.g. shelter) may be more appropriate and better predict patterns of conflict in *N. pulcher*.

In the current study, we observed that breeders were more connected than helpers in dominance and association networks. In addition to investing heavily in deterrent signals directed at large helpers to maintain their social status (see above), breeders may also be more connected than helpers if they use dominance interactions to police the behaviours of, and interactions among, helpers. Pay-to-stay models of cooperative breeding predict that breeders will punish helpers who provide insufficient help (Gaston

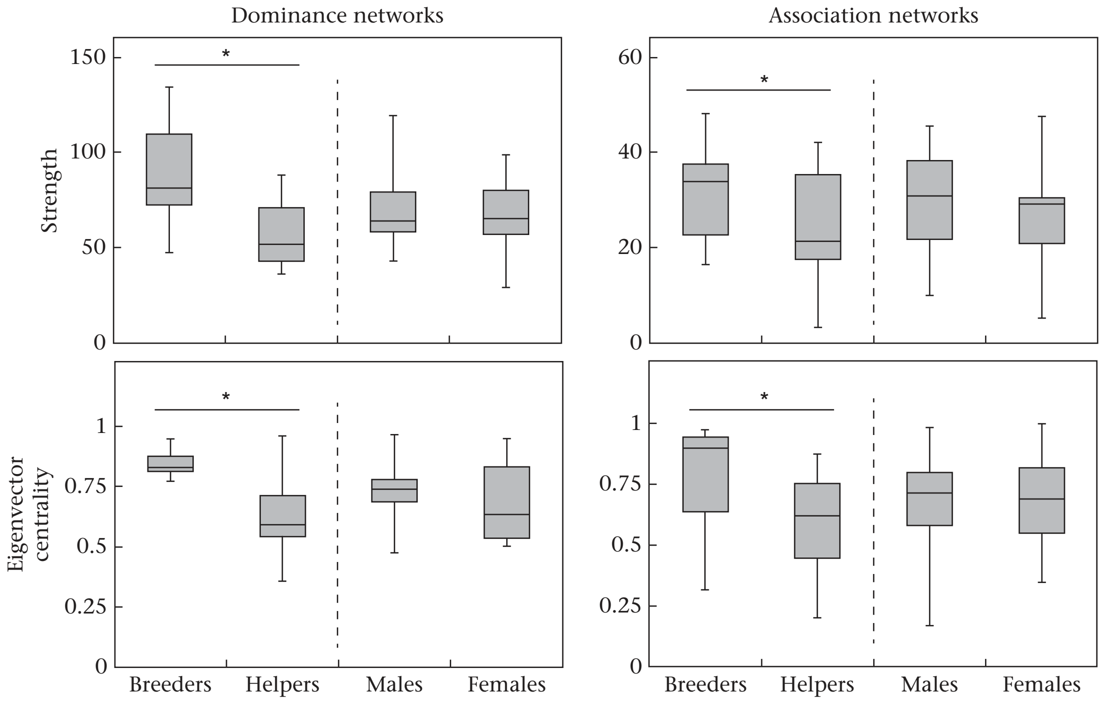

Figure 3. Connectivity in *Neolamprologus pulcher* social networks. Shown are the strength and eigenvector centrality of classes of individuals for networks of dominance interactions and association in 14 captive social groups. Box plots show the 75th percentile, median and 25th percentile, as well as the minimum and maximum values (whiskers). *$P < 0.05$.

1978; Kokko et al. 2002). The evidence for such dominant policing of subordinate helping in *N. pulcher* is weak (Wong & Balshine 2011a). However, it is possible that breeders do police helpers in terms of their shelter or space use. Furthermore, breeders may be more connected if they police interactions among helpers to increase group stability and/or productivity. Third-party policing is thought to be most common in societies with high interindividual variance in power, because policing by high-ranking individuals will be more effective and less costly in this situation (Flack et al. 2005). Size-structured hierarchies in social fish fit these criteria, and there were several observations of breeders interfering in helper–helper conflicts in this study (C. M. O'Connor & A. R. Reddon, personal observation) and in previous field studies (S. Balshine, personal observation). A further comprehensive study of policing in *N. pulcher* social groups is probably warranted.

Finally, we show that patterns of association between *N. pulcher* group members are nonrandom. These patterns were not explained by sex or size asymmetry of group members. One possible explanation is that both in captivity and in the wild, individual *N. pulcher* establish small subterritories within the larger group territory (Werner et al. 2003). Such segregation of space could lead to nonrandom association patterns, because individuals will predominantly associate with neighbours. Subterritoriality was widely observed in this study, however, it seems that the arrangement of subterritories was not dependent on the sex or body size of the individuals in neighbouring subterritories. While captivity will always constrain animals in their ability to move throughout their environment (Estévez & Christman 2006), the aquaria used in this study were a similar size to the median territory size reported for wild *N. pulcher* (Balshine et al. 2001). Thus, the patterns of association observed in this study are unlikely to have been generated by the captive environment alone and may be similar to those in wild populations.

In conclusion, this study is one of only a few to examine network structure in multiple replicate social groups of a given species. This approach facilitates making general conclusions about the social structure in this cooperatively breeding cichlid. We provide the first explicit analysis of dominance hierarchy structure in *N. pulcher*, and confirm the assumption that hierarchies are strongly linear and dominance interactions are directionally consistent. Furthermore, we provide evidence that while dominance interactions and associations occur nonrandomly, they are not related to body size asymmetry or sex. Thus, there is little support for sex-specific dominance hierarchies in *N. pulcher*. Our results do show, however, that there is increased social conflict at the top of the size hierarchy, which is consistent with theoretical predictions based on intensified conflict as the probability of inheriting a breeding position increases. Finally, we demonstrate that breeders are more connected than helpers, which suggests interesting avenues for future research on policing in this species. Taken together our results provide valuable information on the structure of social groups in a model cooperative breeding species. Future research examining how network structure influences reproductive success, growth, survival as well other aspects of group function (e.g. predator defence) will be valuable in gaining a more complete understanding of sociality in this intriguing fish.

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Chapter 8: General discussion

Adam R. Reddon

In this thesis, I provide an integrative perspective on social decision making in an emerging model of social system evolution, the cooperatively breeding cichlid fish, *Neolamprologus pulcher*. In particular, I focus on the topics of sociality and social partner assessment, each of which forms a key building block of complex social systems. I explored these topics from an integrative perspective, examining both social behaviour and the proximate mechanisms that underlie it. At the behavioural level, I have focused on the process of social decision-making, and have described key attributes of the social decision-making process in *N. pulcher* as it pertains to grouping behaviour and resource contests. At the proximate level, I have focused on the role of the highly conserved oxytocic hormone system in regulating social behaviour in *N. pulcher*. Through the combination of these approaches, I provide novel insights into the decision-making processes and physiological foundations that support the social behaviour and group structure that we observe in *N. pulcher*. In the remainder of this discussion, I will expand upon some of the key issues raised by my data chapters, and highlight the insights that my work sheds on the structure of *N. pulcher* groups and on the integrative study of social behaviour general.

1. Sociality and group joining decisions

In Chapter 2 of my thesis I examined the grouping decisions of both male and female *N. pulcher*. I detected a robust sex difference in *N. pulcher,* males consistently selected the larger of two possible groups of same-sex conspecifics whereas females will chose the larger group only when they can join at the same high rank. This result has now been replicated in several additional experiments (Chapter 6; A. Reddon unpublished data) and suggests that males and females value somewhat different things when making group-joining decisions. These differences may have important effects on the composition of observed *N. pulcher* social groups. Because males are the dispersing sex in *N. pulcher* (Stiver et al. 2007), one possible explanation for this sex difference is that males have greater outside options. If males move between groups with more often than females, they may be mostly concerned with remaining safe until they are large enough to challenge for a breeding position because they can switch groups if they find themselves in an unfavorable social situation. Therefore, males may not place as much emphasis on their rank when they are low in the hierarchy and well away from legitimate breeding size. Females, by contrast, rarely switch groups and so may be much more sensitive to protecting, maintaining, and advancing their rank within their current group. Future work looking at group-joining behaviour from the other side, the responses of existing group members to potential joiners (Jordan et al. 2010), should provide important insights into this interesting sex difference in joining preferences. If my hypothesis that sex differences in dispersal tendency drive sex differences in group joining preferences is correct, then females should be especially avid policers of new joiners, particularly of joiners that are near to them in size, and that therefore pose a threat to their rank position. Males, especially those that are not near a highly valuable breeding position, may be more ambivalent or even encourage joiners to gain the antipredator benefits of larger group size (Balshine et al. 2001; Heg et al. 2005).

2. Social partner assessment and decision-making in resource contests

One of the more striking findings in my thesis comes from Chapter 3, wherein I present evidence that decision-making (both tactical and strategic) within *N. pulcher* resource contests is guided by the assessment of opponent fighting ability (estimated here by body size; also known as resource holding power or potential; RHP), but not by the fighting ability of the focal animal itself. It is widely believed or even assumed that animal contests are normally resolved by mutual assessment of fighting ability and that animals compare their own prowess with their estimate of the fighting ability of their opponent before arriving at a decision of whether to withdraw, persist or escalate in a fight (Maynard-Smith & Parker 1976; Enquist & Leimar, 1983; Enquist et al. 1987, 1990; Arnott & Elwood 2009; Briffa & Elwood 2009, 2010; Elwood & Arnott 2012; Riechert 2013). Perhaps because it is intuitively satisfying (Maynard-Smith & Harper 2003), and because it closely mimics the way our own species makes decisions within contests (Sell et al. 2009a,b; 2010), mutual assessment is often assumed to be operating (Taylor & Elwood 2003; Arnott & Elwood 2009; Elwood & Arnott 2012). However, the evidence for this oft-made claim is scant (Taylor & Elwood, 2003; Arnott & Elwood, 2009; Briffa & Elwood 2010). The number of correctly analyzed studies providing strong inferential support for mutual assessment in non-human animals is surprisingly small (Elwood & Arnott 2012).

In Chapter 3, I report the results of a study which joins an increasing number of cases in which the characteristics of the opponent clearly have been shown to have an important effect on decision-making in contests while the fighting ability of the focal animal apparently does not (e.g., Rillich et al. 2007; Prenter et al. 2008; Arnott &

Elwood, 2010; Jennings et al. 2012; Peixoto & Benson 2012). These studies challenge the long held assumption that mutual assessment universally explains decision-making during contests, and novel models, which integrate these empirical findings into a comprehensive theoretical framework, are an important future research goal (Elwood & Arnott 2012; Riechert 2013).

In an effort to move towards a theoretical framework of decision-making during contests that better fits with emerging empirical results, here I present a preliminary verbal model of **'opponent-assessment without self-assessment' (OA-WSA**). In this verbal model, I endeavor to explain why animals may, in certain cases, make decisions within contests based on the fighting ability of their opponent without reference to their own fighting ability. In essence, I combine insights from other researchers (see in particular, Fawcett & Johnstone 2010; Elwood & Arnott 2012) in what I believe is a novel way.

To begin, I assume that the inherent costs of contests can be attenuated, particularly for the loser of a fight, by gathering information about the fighting ability of ones opponent and using that information to guide decisions during a contest. Under strict self-assessment cost threshold models (e.g., Maynard-Smith & Price 1973; Payne & Pagel 1996, 1997; Mesterton-Gibbons et al. 1997; Payne 1998) wherein no information is gathered about opponent strength, losers will always pay their maximum acceptable cost (Arnott & Elwood 2009). By making use of information about opponent fighting ability, the losers of a fight can reduce their costs by giving up before their maximum cost threshold has been reached (Maynard-Smith & Parker 1976; Enquist & Leimar 1983; Enquist et al. 1987, 1990; Maynard-Smith & Harper 2003). This minimization of costs for the loser has led many researchers to take mutual assessment for granted (Taylor & Elwood 2003; Arnott & Elwood 2009; Elwood & Arnott 2012). However, while gathering information about an opponent's fighting ability makes intuitive sense, particularly given that many aggressive signals appear to be designed to accurately convey information about fighting ability (Hurd 1997; Hurd & Enquist 2001; Maynard-Smith & Harper 2003), the gathering of information about ones own abilities is a non-trivial task (Elwood & Arnott 2012). Perceptual limitations on the ability to receive information about the size of ones own signals and hence fighting ability may restrict the degree to which animals know their own strength (Fawcett & Johnstone 2010). For example, a frontal display, where a fish extends its opercula and holds it mouth agape as an advertisement of body size, is useful in conveying information to a conspecific receiver (Baerends & Baerends-Van Roon 1950) but very difficult for the signaler itself to perceive. Furthermore, even if the focal animal did have clear information about its own size, strength, or fighting ability, the comparison process between two uncertain quantities (i.e., own and opponent fighting ability) is a cognitively demanding and sophisticated task that is unlikely to be present in many taxa (Elwood & Arnott 2012).

In the OA-WSA model, I suggest that: 1) animals do not receive information from their own signals; 2) as a result very little information about ones own fighting ability is gained during the course of a contest and 3) the perception of ones own fighting ability is updated between contests and not within them. Under OA-WSA, the focal animal will compare an estimate of its own fighting ability that is based on its previous experiences to its assessment of the strength of its opponent. The results of previous contests (also known as winner and loser effects) can have a profound and lasting influence on future contest behaviour (Dugatkin et al. 1997; Hsu et al. 2006). My verbal model builds upon the insights of another model of contest behaviour, which did not include any opponent assessment, but did show that updating an estimate of one's own fighting ability based on winner and loser effects is plausible (Fawcett & Johnstone 2010). Rather than making an assessment of ones own strength during a contest, an animal's perception of its own fighting ability is updated in a Bayesian manner between contests by integrating winner and loser status (Hsu et al. 2006) with the information gained about the fighting ability of opponents (Maynard-Smith & Parker 1976; Enquist & Leimar 1983; Arnott & Elwood 2009; Riechert 2013). For example, a loss to a weak opponent suggests that the focal animal is weak, whereas a win against an strong opponent suggests that the focal animal is strong. Through successive contests, the focal animal can increasingly zero in on its own true fighting ability and behave accordingly in the future. The animal should place greater emphasis on the results of more recent contests to allow for developmental/life-history changes in fighting ability, injuries, practice effects, etc., which may have lasting effects on fighting ability.

The second component of my verbal model draws from recent work by Elwood and Arnott (2012), suggesting that animals need not make cognitively demanding comparisons between their own and their opponents fighting ability, but that each of these pieces of information can act as discrete inputs with positive and negative weightings respectively. These inputs are then combined in an additive way to create a behavioural output (e.g., to give up a retreat from the contest). If an animal believes its own fighting ability to be high prior to a contest, this will increase aggression and persistence whereas if an animal evaluates its opponent as being strong during a contest, this will decrease aggression and persistence. Inherent costs (e.g., energy, time, injuries, the risk of predator attack) will accumulate over the course of a contest driving the focal animal towards its cost threshold (i.e., the point at which an animal withdraws from a contest). Evaluation of own strength (based on previous results of previous fights) and of opponent strength (actively gathered by received aggressive signals from the opponent animal) will be added to the accumulating costs of the fight to arrive at a behavioural decision. By including this simplified decision-making system, the OA-WSA model has the benefit of sidestepping the problem of the unrealistic cognitive demands of making a direct comparison between two subtly different and uncertain quantities (i.e., own and opponent fighting ability; Elwood & Arnott 2012).

My OA-WSA model makes several novel and testable predictions about the nature of animal contests including: 1) animals gain knowledge about their own fighting ability through fighting. Therefore, animals with little fighting experience will have poor information about their own strength and therefore behave in a manner that is consistent with opponent-only assessment. 2) As animals gain fighting experience, they will update their estimate of their own fighting ability by integrating information about the outcome of their previous contests and the strength of their opponents in those contests. As a result, animals should fight in a way that increasingly converges on the predictions of mutual-assessment as their fighting experience increases and they get a better estimate of their own fighting ability. Finally, 3) contests have informational value in allowing the animal to refine its estimate of its own fighting ability. In this way, fighting is inherently useful, win or lose, as it provides valuable information that can be used to optimize future behaviour. Therefore, inexperienced animals may be more willing to fight as the added information is of the greatest utility to them, whereas highly experienced animals may be more reticent to engage in contests they are not confident in winning as the benefit of increased information is less of an offset for the inherent costs of fighting (Fawcett & Johnstone 2010).

The results of Chapter 3 suggest that OA-WSA may explain fighting behaviour in *N. pulcher*, as the fish in that study seemed to make decisions based only on their opponents perceived fighting ability. In Chapter 4, I found that smaller *N .pulcher* fish were more motivated to return to a contest following a simulated predator attack. If the smaller fish in that study were younger and or less experienced in fighting, then this result would be supportive of prediction 3 of OA-WSA. Fish age and size are typically highly correlated, due to indeterminate growth (Bone & Moore 2008).

I think that the OA-WSA model may provide a useful addition to the literature on animal contests by providing a plausible explanation for the paradoxical finding that animals sometimes seem to use information about their opponent's fighting ability but not their own fighting ability when making decisions in contests. Future theoretical and empirical work will help to illuminate the details of this model and help to determine how much predictive utility the model has in explaining contest dynamics. Strong tests of the predictions of OA-WSA will require long-term studies with good experimental and life history control over the study species (see Arnold & Taborsky 2010; Taborsky et al. 2012a,b for examples in *N. pulcher*). I believe that such studies are a worthwhile next step, as the current models of assessment in contests fail to explain many empirical observations. *N. pulcher*, given their small body size (Taborsky 1984), rapid development (Arnold & Taborsky 2010) and amenability to aggression research (Desjardins et al.

2005; Taves et al. 2009; Riebli et al. 2011 Chapters 3, 4, 5 and 7 of this thesis) make them good candidate system to further investigate OA-WSA. Explicit mathematical modeling of the OA-WSA verbal model I present here will also be invaluable.

3. Oxytocic regulation of behaviour

I found mixed evidence for the hypothesis that the oxytocic hormone system is a key regulator of social behaviour in *N. pulcher*. On one hand, in Chapter 5, I found evidence that isotocin treatment affects some aspects of *N. pulcher* behaviour, including the assessment of opponent characteristics during aggressive interactions and the response to aggressive challenges from dominant group members within naturalistic social groups. On the other hand however, in Chapter 6 we found that sociality, or the tendency to form groups with conspecifics, was only modestly affected by treatment with either isotocin, or a specific isotocin receptor antagonist. The effects that I found in Chapter 6 were also not in the predicted direction. I expected that isotocin augmentation would increase social motivation but that did not happen. Taken together, these results suggest that isotocin in *N. pulcher* plays a complex and context-dependent role in regulating social behaviour. These results join a growing body of evidence that calls into question the naïve characterization of oxytocin and its non-mammalian homologues as generalized promoters of prosocial behaviour (e.g., MacDonald & MacDonald 2010; van Anders et al. 2011).

In a particularly incisive critique, Churchland and Winkleman (2012) suggest that the majority of oxytocin studies fail to rule out an anxiolytic effect as a more parsimonious explanation of the seemingly selectively social effects of oxytocin that are often reported. An anxiolytic perspective may help to explain some of the results I found in manipulating the isotocin system in *N. pulcher*. First, in Chapter 5, IT-treated fish were more aggressive to larger opponents, while saline-treated fish behaved in accordance with their own intrinsic aggressive propensity. While this result suggests an increased use of social information (i.e., increased assessment of opponent characteristics) in making social decisions, the effect went in the opposite direction to what I would have predicted based on the results of Chapter 3, where fish were less motivated to fight against large opponents. An anxiolytic effect of isotocin could explain this unexpected result. If the isotocin treatment attenuated fear in the treated fish, this could have caused them to be more aggressive when competing with more formidable opponents. However, the results of the second experiment in Chapter 5, where the fish showed greater submission to larger group members, does not seem to fit with the idea that isotocin is merely reducing stress or fear. In Chapter 6, I show that certain doses of isotocin reduced sociality rather than increasing it (in contrast to our initial prediction), and that a selective isotocin receptor antagonist increased sociality but only in males and not in females. Grouping behaviour is a well-known stress-coping mechanism in other species of fish (Lima & Dill 1990; Krause & Ruxton 2002, 2010) but grouping in response to stress has not yet received much attention in *N. pulcher*. In an unpublished laboratory experiment, I presented *N. pulcher* with a choice between a group of three same sex conspecifics or a lone same sex individual (as in Chapter 2) after treating the water with an olfactory chemical alarm cue that simulated a recent predator attack (Chivers & Smith 1998; Wisenden et al. 2004; Chivers et al. 2007; Reddon & Hurd 2009) or a blank water control. I did not find an increased grouping response in *N. pulcher* using this experimental protocol (A. Reddon unpublished data). Similarly, in another unpublished study from our laboratory, the visual presentation of a live predator (*Lepidiolamprologus lemairii*) did not increase sociality in *N. pulcher* (C. O'Connor et al. unpublished data). If *N. pulcher* do respond to stressful situations with increased grouping behaviour, then a putative anxiolytic effect of isotocin could fit with our findings as reduced stress could reduce the grouping response and so increased anxiety caused by the isotocin receptor blocker could induce increased grouping. However, currently there is not much evidence to suggest that *N. pulcher* group in response to stress and more work on this topic is clearly needed.

In a recent review of nonapeptide regulation of sociality across taxa, Goodson (2013) emphasizes that the current state of the evidence on the function of nonapeptides (including oxytocin/isotocin), in regulating sociality, is uncertain at best due to the restricted set of species for which we have good data and the strong effects of both sex and social context on the observed behavioural effects. My thesis contributes additional data to this body of work, and emphasizes further that the relation between oxytocin and its non-mammalian homologues (including isotocin) and social behaviour remains unclear. Clearly, further work in a greater diversity of taxa and social systems will be essential in uncovering the precise role of the oxytocin family of nonapeptides in regulating social behaviour. Work on fish should be valuable in unraveling the functions of the oxytocic system in regulating social behaviour (Godwin & Thompson 2012). Explicitly comparative approaches will also be essential in making sense of this complexity (see Goodson & Kingsbury 2011; Goodson 2012b for descriptions of the most comprehensive comparative work on the social regulatory functions of nonapeptides available to date).

Lamprologine cichlids, including *N. pulcher*, offer enormous potential as a comparative system because of the remarkable evolutionary radiation of these fishes (Barlow 2002) and the increasing availability of molecular techniques (Aubin-Horth et al. 2007; Renn et al. 2008). Therefore this group may be of great utility in uncovering the functions and evolutionary significance of nonapeptide regulation of sociality and the divergence social systems. *N. pulcher* has several close relatives that offer an excellent comparative opportunities (Day et al. 2007). *Telmatochromis temporalis*, for example, lives in the same areas, uses the same resources, and has a similar breeding system to *N. pulcher* (Kuwamura 1986, Mboko et al. 1998). However, *T. temporalis*, are less social, never show cooperative breeding and never form groups, rarely interacting with conspecifics outside of breeding and resource contests (Mboko & Khoda 1999; Heg & Bachar 2006). A third lamprologine species, which is a related to both *N. pulcher* and *T. temporalis* (Day et al. 2007), *Neolamprologus caudopunctatus*, is also endemic to the same areas of Lake Tanganyika as *N. pulcher* and *T. temporalis* and also reliant on the same food and territorial resources (Konings 1998). Like *N. pulcher* and *T. temporalis*, *N. caudopunctatus* is a pair-breeding substrate spawner (Demus 2012). *N. caudopunctatus*, however, represents an intermediate level of social complexity between the highly social *N. pulcher* and the much less social *T. temporalis* (Schaedelin et al. 2012; 2013). *N. caudopunctatus* do not engage in cooperative breeding or form rigid linear dominance hierarchies like *N. pulcher*, but do form large social aggregations and show a high degree of sociality motivation that is absent in *T. temporalis* (Demus 2012). This three-way comparison would allow the disentangling of sociality and cooperative breeding, while controlling for ecology and mating system, which are factors highly interrelated with nonapeptide functioning (Goodson & Kingsbury 2011; Goodson 2013). Collectively, these three species provide a close corollary to the exceptional comparative work that the Goodson research group has done with estrildid finches (Goodson et al. 2009; Goodson & Kingsbury 2011; Goodson 2012b) and offer an excellent opportunity to independently investigate the role of the nonapeptides, including isotocin, in regulating sociality, social complexity, and social system evolution. The work on isotocin in *N. pulcher* I present in this thesis lays some of the groundwork for future comparative studies of nonapeptide regulation of social behaviour in lamprologine cichlids. Ongoing comparative work in our laboratory on *N. pulcher* and *T. temporarlis* has already begun to contribute to our understanding of social system evolution and the role nonapeptides play in regulating social behaviour in this group of fishes (Hick et al. in press; O'Connor et al. in prep).

4. Social organization

My thesis presents several key insights about the structure and organization of social groups in *N. pulcher*, which may be generalizable to other small-scale hierarchical animal societies. In particular, Chapter 7, which was designed explicitly to test several hypotheses about group level social interactions in *N. pulcher*, provides several interesting insights. First, I confirmed empirically for the first time the long-held belief that *N. pulcher* groups are organized as highly linear size-based dominance hierarchies (Taborsky 1984; Balshine et al. 2001; Wong & Balshine 2011a,b). I also report in Chapter 7 that conflict within *N. pulcher* social groups escalates with increasing rank

(Wong & Balshine 2011a,b). This result is in line with the predictions of a recent model of social organization within hierarchical societies with high reproductive skew, whereby the value of social rank as a defensible resource increases exponentially up the hierarchy (Cant et al. 2006; Buston & Cant 2006). The results of Chapter 7 do, however, clearly contradict another widely held belief about *N. pulcher* social structure, which is the idea that there are separate male and female size-specific linear dominance hierarchies within the social group (Taborsky 1984, Balshine-Earn et al. 1998; Balshine et al. 2001). Our results show that male and female *N. pulcher* in the same group form one single mixed-sex dominance hierarchy, and there is no sex-specific targeting of dominant/subordinate interactions. This is fitting with some other results of my thesis, namely, that male and female *N. pulcher*, show few sex differences in behaviour, especially in the context of aggression (Chapters 3, 4, 5, 7).

N. pulcher dominance hierarchies are strongly size-based (Chapter 7). Interestingly, observations in the wild suggest that natural *N. pulcher* groups tend to maintain approximately a 5% difference in body size between each pair of rank-adjacent group members, and individuals who encroach on this threshold are often evicted from the group (Taborsky 1984; Heg et al. 2004, 2010; Hamilton et al. 2005; Zottl et al. 2013). The 5% value is interesting because that is precisely the threshold I detected for resource contests between pairs of *N. pulcher* to be decisive in the vast majority of cases. Contests between *N. pulcher* that are more than 5% different in body size, are nearly always won by the larger fish, whereas in fights between fish that are less than 5% different in body size the winner cannot be predicted *a priori* (Chapter 3). Together, this suggests that *N. pulcher* may be actively maintaining this 5% difference in body size, either through selfimposed growth restriction to avoid conflict, or top-down suppression of growth from dominant group members in order to maintain a stable dominance hierarchy in which all fish can decisively dominate all group members below them in rank (Buston 2003; Heg et al. 2004, 2010; Hamilton et al. 2005; Wong et al. 2007, 2008). Further work into the mechanisms of this size stratification within *N. pulcher* groups will provide important insights into the basis of social organization in this species and perhaps other hierarchical animals societies.

5. Conclusions

In this thesis, I investigated social decision making, with a focus on aggressive and affiliative decisions in an emerging model system for the integrative study of social behaviour, the highly social cichlid fish, *Neolamprologus pulcher*. I explored the role of the nonapeptide hormone isotocin (the teleost fish homologue of oxytocin) as a proximate regulator of decision-making during contests, within naturalistic social groups and during social group preference tasks. I found evidence that *N. pulcher* have a high sociality motivation, preferring to associate with larger groups of conspecifics to small ones and that this motivation is stronger and more general in males. I also found that *N. pulcher* pay attention to opponent characteristics during resource contests, but appear not to fight with regard to their own strength. Finally, I found mixed evidence for the hypothesis that isotocin is a key regulator of social decision making in this species. Isotocin appears to increase attention to social information but does not increase and may in fact decrease grouping motivation in *N. pulcher*. Taken together, my doctoral work helps to elucidate the behavioural and hormonal factors underlying decision-making in the context of aggression and sociality in a cooperatively breeding vertebrate. My thesis informs the debate on the evolution of social behaviour, enriching our understanding of the emergence of complex social systems by examining key building blocks of social organization.

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