Social motivation and conflict resolution tactics as potential building blocks of sociality in cichlid fishes

Sigal Balshine[1], Marian Y. L. Wong[2], Adam R. Reddon[3*]

[1] *Department of Psychology, Neuroscience, and Behaviour, McMaster University, 1280 Main Street West, Hamilton, Ontario, L8S 4K1, Canada*

[2]*School of Biological Sciences, University of Wollongong, Northfields Avenue, Wollongong, NSW 2522, Australia*

[3]*Department of Biology, McGill University, 1205 Ave, Docteur Penfield, Montreal, QC, H3A 1B1, Canada*

* *Corresponding author: adam.reddon@mcgill.ca*

ABSTRACT

Even closely related and ecologically similar cichlid species of Lake Tanganyika exhibit an impressive diversity of social systems, and therefore these fishes offer an excellent opportunity to examine the evolution of social behaviour. Sophisticated social relationships are thought to have evolved via a building block design where more fundamental social behaviours and cognitive processes have been combined, incrementally modified, and elaborated over time. Here, we studied two of these putative social building blocks in two closely related species of cichlids: *Neolamprologus pulcher* a group-living species, and *Telmatochromis temporalis*, a non-grouping species. Otherwise well matched in ecology, this pair of species provide an excellent comparison point to understand how behavioural processes may have been modified in relation to the evolution of sociality. Using social assays in both the laboratory and in the field, we explored each species' motivation to interact with conspecifics, and each species' conflict resolution tactics. We found that individuals of the group living species, *Neolamprologus pulcher*, displayed higher social motivation and were more likely to produce submission displays than were individuals of the non-grouping species, *Telmatochromis temporalis*. We argue that the motivation to interact with conspecifics is a necessary prerequisite for the emergence of group living and that the use of submission reduces the costs of conflict and facilitates the maintenance of close social proximity. These results suggest that social motivation and conflict resolution tactics are associated with social complexity, and that these behavioural traits may be functionally significant in the evolution and maintenance of sociality.

Keywords: cooperative breeding; sociality; group living; aggression; submission; Lake Tanganyika

1. Introduction

Sociality is not a single cohesive unit of behaviour, but instead is comprised of a diverse set of socially relevant actions and cognitive processes (Goodson, 2013). Complex social behaviours are thought to have evolved from a combination of basic behavioral units. Examples include the tendency to approach conspecifics, recognition and discrimination of individuals, and the use of tactics to resolve conflicts at minimal cost (Soares et al., 2010). Small behavioural changes, mediated by subtle alterations in the underlying physiological machinery, are gradually added and modified to form complex social phenotypes (Goodson, 2005; Donaldson and Young, 2008; Soares et al., 2010; O'Connell and Hofmann, 2011; Zayad and Robinson, 2012). Therefore, in order to understand the emergence of complex social behaviour and group living lifestyles, it is necessary to understand how these basic behavioural building blocks have changed in form and function during the divergence of social systems.

The explosive radiation of the African cichlid fishes has generated an impressive diversity of species with considerable variation in morphology, ecology, and behaviour and has made this family a classic ecological, evolutionary and behavioural model system (Meyer et al., 1994; Barlow, 2000; Kocher, 2004). The lamprologine cichlid tribe of Lake Tanganyika, East Africa, shows particularly remarkable diversity in social behaviour among its more than 80 species (Kuwamura, 1986; Konings, 1998; Day et al., 2007; Sturmbauer et al., 2010). As a result, this group offers excellent opportunities for comparative social behaviour research. Of special note, the lamprologine cichlids count amongst their ranks all known cooperatively breeding fishes (Taborsky and Limberger, 1981; Taborsky, 1994; Heg and Bachar, 2006). These cooperative species live in relatively permanent social groups in which non-breeding subordinates assist the dominant breeding pair in their reproductive efforts. A high level of social complexity characterizes cooperative breeding societies, with group members that interact frequently and have individualized relationships (Freeberg et al., 2012; Dey et al., 2013). Cooperative breeding has emerged multiple times among the lamprologine cichlids and is derived from the pair breeding system typical for cichlids (Dey et al., in review), in which adult fish are generally intolerant of other conspecifics other than their own mate (Kuwamura, 1986; Desjardins, et al., 2008).

In order to better understand the behavioural building blocks of sociality, we investigated socially relevant behavior in two closely related lamprologine cichlids, *Neolamprologus pulcher* and *Telmatochromis temporalis* (Figure 1). These two species split approximately 2 million years ago (Day et al., 2007; Sturmbauer et al., 2010) and continue to share a similar ecology, but have diverged dramatically in their social system. *Neolamprologus pulcher* are cooperative breeders that live in permanent social groups consisting of a single dominant breeding pair, and an average of 5-7 subordinate fish that act as helpers at the nest, assisting with brood care, territory maintenance and defence (Taborsky and Limberger, 1981; Taborsky, 1984; Balshine-Earn et al., 1998; Balshine et al., 2001; Heg et al., 2005; Wong and Balshine, 2011). These subordinate group members are often not closely related to the dominant breeding pair (Stiver et al., 2004, 2005, Hellman et al., 2015). In contrast, *T. temporalis* never form groups (Mboko and Khoda, 1999; Heg and Bachar, 2006). However, both species live in the same areas of the rocky littoral zone in Lake Tanganyika and share similar habitat requirements and predation regimes (Kuwamura, 1986; Brichard, 1989; Konings, 1998). Furthermore, both cichlids are territorial substrate spawners with biparental care (Kuwamura, 1986). Both species are small bodied (<80 mm standard length) and readily adapt to the laboratory environment.

Using these two species (one group living and one not), we measured and compared behaviours hypothesized to be building blocks of sociality (Soares et al., 2010). Using newly collected data from the laboratory and the field, we examined social motivation, the tendency to value interactions with conspecifics compared to other alternatives. Additionally, by reanalyzing previously published data, we tested conflict resolution tactics that are used to settle an agonistic interaction. We predicted that relative to the non-grouping *T. temporalis*, the group-living *N. pulcher* would display greater social motivation and make greater use of submissive behaviour, a conflict resolution tactic that facilitates group formation and maintenance (Bergmüller and Taborsky, 2005). Through this set of studies, we hoped to gain insight into some of the basic behavioural building blocks that make up a highly social phenotype, and broaden our understanding of the evolution and maintenance of sociality.

2. Methods

2.1. Measurement of social behaviour in the field

Field based behavioural studies were conducted at our long-term study site located at Kasakalawe Bay (8°46'52" S, 31°5'18" E) in Lake Tanganyika, Zambia. This site is characterized by mixture of sand and cobble substrate with a gentle descent to depth (for detailed descriptions of the study site, see Balshine-Earn et al., 1998; Balshine et al., 2001; Stiver et al., 2005; Bergmüller et al., 2005; Dierkes et al., 2005; Heg et al., 2005). We performed underwater behavioural observations at depths of 8-12 m using SCUBA. All of the wild fish included in the current study were observed between October-December 2008. To control for ecological conditions, 10 *T. temporalis* territories and 10 *N. pulcher* territories were located such that pairs of territories (one belonging to each species) were within 2 m of each other and were observed on the same day. Two 10 min focal observations (one in the morning and one in the afternoon) were conducted on each breeder in each selected territory. During the observation periods, we recorded all behaviours performed by and directed towards the focal individual. Dominant breeding individuals are easily identified for both species, as the dominants are typically the largest individuals in the *N. pulcher* group and the only fish in *T. temporalis* territories (Wong and Balshine, 2011). Observed *N. pulcher* groups ranged in size from 4 to 9 adult sized individuals (mean ± S.E.M. = 6 ± 0.4. The behaviours recorded are detailed in published ethograms for *N. pulcher* and *T. temporalis* and were based on extensive behavioural observations of males and females of both species in the field and in the laboratory (Sopinka et al., 2009; Hick et al., 2014; Reddon et al., 2015). Briefly, behavioural acts and displays recorded included aggressive, submissive, affiliative, workload, and self-maintenance behaviours. Aggressive behaviours included head-down postures and frontal displays, as well as overt aggressive acts with physical contact, such as chases, rams, bites, or mouth wrestles. Submissive behaviours are typically produced in response to aggressive behaviour from another individual, and consist of head-up submissive postures and quivering submission displays (Reddon et al., 2015). Affiliative behaviors are spontaneously produced towards another individual, and include behaviours such as swimming closely in parallel, and soft touches. Workload behaviors included territory defence, maintenance and offspring care. Finally, self-maintenance behaviors such as feeding and scraping were also recorded. The frequencies of these behaviors from the morning and afternoon observations were averaged for each individual. From these field observations on wild fishes, we calculated the following measures of social investment: 1) total social behaviour performed (the sum of all aggressive, submissive, and affiliative behaviours performed by each focal individual); and 2) proportion of social behaviour performed (total social behaviour divided by the sum of all behaviours performed). To normalize this field data by the opportunity for social interactions, we divided the number of interactions observed by group size (always n=2 for *T. temporalis* breeders but variable for the *N. pulcher* breeders, n = 4-9).

2.2. Measurement of laboratory behaviour

Laboratory-based behavioural studies were conducted between May-November 2012 at McMaster University in Hamilton, Ontario, Canada. The *N. pulcher* and *T. temporalis* used were laboratory-reared descendants of wild-caught fish. All fish were sexually mature but had not yet reproduced. The fish used were measured, weighed, sexed by examination of the external genital papillae, and each was given a unique dorsal fin clip for identification on the day prior to the behavioral trial. These fin clips do not affect the behaviour of the fish and grow back within a week or two (Stiver et al., 2004). Focal fish of each species were housed in 189 L aquaria in mixed sex groups of 8 to 12 individuals (approximately equal numbers of males and females). Both housing and test aquaria contained 3 cm of coral sand substrate, a water filter, heater, and a thermometer. Housing aquaria also contained 2 flowerpot halves for use as shelters. The water temperatures of all aquaria were held at 26 ± 2ºC. All fish were fed dried prepared cichlid food *ad libitum* six times per week, and kept on a 13:11 light:dark cycle.

2.3. Social approach assay

To assess the basic social motivation of each species, fish were placed in a 189 L experimental tank (Figure 2). Sample sizes were n=20 individuals per species, with equal numbers of males and females tested. A conspecific stimulus fish, always of the same sex, and unfamiliar to the focal fish (i.e., from a different housing aquarium) was used. This stimulus fish was between 5-20% larger by mass than the focal individual (Reddon et al., 2011a). The focal fish was initially placed within a perforated transparent cylinder (11 cm diameter) in the center of the tank for 10 minutes. During this acclimation period the focal fish could see an unfamiliar conspecific in an identical cylinder on one side of the aquarium, and a shelter (an opaque black PVC tube; 6.5 cm diameter x 10 cm length) on the other side of the aquarium (Figure 2a). These tubes are readily used as shelter and nesting sites by both species and fish will vigorously fight for access to them (Reddon et al., 2011b; Hick et al., 2014). As result, this test creates a conflict between two potentially rewarding stimuli, the opportunity to interact with an unfamiliar conspecific and access to a desirable shelter. Placement of the unfamiliar fish versus the shelter on the left or right side of the apparatus was determined randomly by coin toss. Following the 10 minutes of acclimation, the central transparent cylinder was lifted remotely by means of a pulley system, releasing the focal fish (Figure 2b). During the 15 minute trial, we then measured social motivation versus motivation to use the shelter in three different ways: 1) initial preference or approach (i.e., whether the fish first approached the conspecific or the shelter); 2) time spent *near* each stimulus (i.e., within 10 cm of either the conspecific or the shelter; a distance that is approximately equal to 2 body lengths of the average focal fish); and 3) time spent *in association* with each stimulus (i.e., interacting across the barrier with the conspecific in the transparent cylinder or using the shelter). The interactions with the stimulus fish consisted primarily of rapid swimming into the cylinder directed at the stimulus fish in an apparent effort to access the other fish.

2.4. Conflict resolution assay

To assess how the two cichlid species differed in terms of their conflict resolution behaviour, we reanalyzed data initially presented in Hick et al., (2014) by focusing on conflict resolution tactics between unfamiliar fish. Full methodological details can be found in Hick et al., (2014), however, in brief: Focal fish were placed with a same-sex conspecific in a 38 L experimental aquarium (Figure 3) and allowed to compete over a shelter for 30 minutes. The fish were given a 1-hour acclimation period prior to the interaction during which they were restricted to a third of the experimental aquarium on opposite ends of the tank and were unable to see the middle or other end chamber. Solid opaque dividers separated the fish from the middle chamber of the aquarium, and from each other. An opaque black tube, identical to the one used in the social approach assay, was placed into each third of the test apparatus (Figure 3a). The solid opaque dividers and the two end shelters were removed after the acclimation period, allowing the two fish to interact and compete over the remaining shelter in the center third of the tank (Figure 3b). This staged sequence reliably elicits a resource contest in both species (Desjardins et al., 2005; Taves et al., 2009; Reddon et al., 2011b). Competitors were always unfamiliar fish that came from different housing aquaria. We ensured that one fish was always 5-20% heavier than its competitor, as this size difference reliably elicits contest behaviour but also allows the eventual winner to be predicted *a priori* (Reddon et al., 2011b). In total, 35 pairs (i.e., 70 fish) were used, with 9 male pairs for each species, 9 pairs of female *N. pulcher* and 8 pairs of female *T. temporalis*. Trials were scored live. All aggressive and submissive behaviours performed by both the eventual loser and the eventual winner were recorded during each 30 min trial. We assigned loser status to any fish that ceased aggressing against its rival and displayed submission or fled from the other fish three times in succession (Reddon and Hurd 2009; Reddon et al., 2011b). Because acts of submission and fleeing are commonly observed in direct response to aggressive behaviour, we divided the rates of submission and fleeing by the number of aggressive acts received (following the measures used in Reddon et al., 2012; O'Connor et al., 2013).

2.5. Statistical analyses

Statistical analyses were conducted using IBM SPSS Statistics Version 23. We compared the species in their social motivation in the field using generalized linear mixed models fitted to a gamma distribution with a log link, appropriate for positively skewed values. We included species and sex as fixed factors and breeding pair identity as a random factor. Species differences in social motivation and conflict resolution tactics in the laboratory were examined using ANOVA for continuous dependent variables. We included species, sex and their interaction as fixed factors in these models. In order to assess which stimulus the focal fish approached first in our laboratory social motivation assay, we conducted a binary logistic regression with first stimulus visited (shelter vs. conspecific) as the response variable, and species, sex and their interaction as fixed factors. The relative use of fleeing compared to submission as a conflict resolution tactic for each species was further explored using ANCOVA with submission per aggressive act received set as the response variable, flees per aggressive act received as continuous covariate with species, sex and their interaction as fixed factors. We checked the residuals from all reported models for adherence to model assumptions and transformed the raw data where appropriate (indicated below). In the majority of our models, sex was not a statistically significant factor ($p > 0.05$), however we do note below those cases in which males and females showed different patterns of behaviour.

2.6. Ethical note

All laboratory trials were continually monitored. Following the recommendations of Huntingford (1984) we minimally handled each fish, and limited the contests to a short duration. The fish were not overtly stressed by the contest and no signs of injury (torn fins or missing scales) were observed during any of the trials. Had any such injuries been sustained, the trial would have been stopped immediately. Neither species is threatened, nor endangered, and are both extremely abundant at our study site. The methods described for animal housing, handling and observations in the laboratory and in the field were assessed and approved by both the Animal Research Ethics Board of McMaster University (Animal Utilization Protocol numbers 06-10-59 and 10-11-71) and the Zambian Department of Fisheries. All procedures adhered to Canadian and Zambian laws, and the guidelines of the Canadian Council for Animal Care and the Animal Behavior Society/Association for the Study of Animal Behaviour.

3. Results

3.1. Social motivation

In the wild, *N. pulcher* were involved in approximately 3x more social interactions than *T. temporalis* (Generalized linear mixed model: $F_{1,36} = 13.91$. $p = 0.001$; Figure 4a). Females of both species performed more total social behaviours than did males (Generalized linear mixed model: $F_{1,36} = 18.84$, $p < 0.001$). Social interactions also made up a higher proportion of all behaviours in the group living species when compared with the non-grouping species (Generalized linear mixed model: $F_{1,36} = 4.63$, $p = 0.038$; Figure 4b), demonstrating that *N. pulcher* breeders spend more of their time budget socializing. After controlling for group size, dominant *N. pulcher* did not show more social interactions than *T. temporalis* (Generalized linear mixed model: $F_{1,36} = 1.68$, $p = 0.20$; Figure 4c). For complete results, see Supplemental Table 1.

In the laboratory assessment of social motivation, there was no clear tendency for fish to approach the conspecific or the shelter first, nor was there a species difference in which stimulus was approached first (Binary logistic regression: Wald $\chi^2 = 1.96$, df = 1, p=0.16, Figure 5a). However, there was a sex difference, with males of both species more likely to approach the fish first while females were more likely to approach the shelter first (Binary logistic regression: Wald $\chi^2 = 6.15$, df = 1, p = 0.013). Both species spent about the same amount of time within 10 cm of the conspecific (ANOVA: $F_{1,36} = 1.16$, p = 0.29, Figure 5b). However, members of the group living species (*N. pulcher*) spent more time interacting with the conspecific than did individuals of the non-grouping species (*T. temporalis*; ANOVA: $F_{1,36} = 5.53$, p = 0.024; Figure 5c). For complete results see Supplemental Table 2.

3.2. Conflict resolution

In the staged contests over a shelter in the laboratory, we found that *N. pulcher* fights contained fewer aggressive acts relative to the contests among *T. temporalis* (Log transformed data; ANOVA: $F_{1,31} = 14.87$, p = 0.001; Figure 5a). The *N. pulcher* were also far more likely to use submission displays to terminate a resource contest with an unfamiliar conspecific (Log transformed data, ANOVA, $F_{1,31} = 8.56$, p = 0.006; Figure 5b), while *T. temporalis* were more likely to flee (ANOVA, $F_{1,31} = 4.37$, p = 0.045; Figure 5c). Across both species, there was a strong negative relationship between the individual tendency to perform submission displays and the tendency to flee from their opponent (ANCOVA, $F_{1,30} = 16.44$, $p < 0.001$; Figure 5d). For complete results see Supplemental Table 3.

4. Discussion

By studying two closely related species of cichlids (Day et al., 2007; Sturmbauer et al., 2010) that are well matched in terms of their habitat requirements, diet, and ecology, but that differ in their social system (Kuwamura, 1986; Heg and Bachar, 2006), we can examine how behavioral processes and cognition may have diversified in relation to sociality. We found that in both the laboratory and the field, individuals of the group-living species, *N. pulcher*, are more motivated to interact with conspecifics. In the laboratory, *N. pulcher* also use submission more frequently to end conflicts when compared to the non-grouping *T. temporalis*.

In the wild, *N. pulcher* have more social interactions than the less social *T. temporalis*. Thus, individuals of the more social species invest a greater proportion of their time budget engaged in social interactions than the non-grouping *T. temporalis*. However, it can be argued that the greater number of social interactions observed in wild *N. pulcher* are due at least in part to the greater opportunity to interact with conspecifics because of the group living situation. Indeed, when we controlled for group size, the *N. pulcher* no longer show significantly more social interactions per group member (4-9 individuals) that did the *T. temporalis* (always 2 individuals). However, the pattern of the results suggests that *N. pulcher* may interact more than *T. temporalis* after controlling for group size, but a larger sample size is needed to resolve this issue. Controlling for group size in this way is also not without caveats, given that interactions within *N. pulcher* groups are strongly size dependent, and individuals that are very different in body size rarely interact (Dey et al. 2013). Larger groups are more likely to contain numerous small helpers that seldom interact with the large dominant individuals that we observed, therefore potentially creating the misleading impression that fish in larger groups interact less after accounting for their apparent opportunity to do so. We argue that the fact that *N. pulcher* spend a greater proportion of their time interacting socially in the face of other competing motivations (e.g., foraging, territory maintenance etc.) than do the non-grouping *T. temporalis* does support the notion that *N. pulcher* are more socially motivated. Concordant with this argument, *N. pulcher* spent more time interacting with a conspecific compared to *T. temporalis* during a standardized preference trial in the laboratory. The tendency to interact with conspecifics is among the most fundamental aspects of social behavior. Without the motivation to remain close to other individuals, no other more complex social interactions are possible (Thompson and Walton, 2004; Soares et al., 2010; Goodson, 2013).

The conflict resolution tactics used by *N. pulcher* are likely to aid in the formation and maintenance of stable social groups. *Neolamprologus pulcher* were more prone to use submission displays than were *T. temporalis*. Conversely, *T. temporalis* were much more likely to flee from a conflict. Our laboratory results indicate that fleeing and submission may be alternative tactics for ending a conflict, and the use of these different approaches to giving-up appear to trade off against each other in both species. Submissive behaviour in general allows competitors to settle a conflict, minimize the costs of fighting (e.g., energy, time and injury risk Mastumura and Hayden, 2006), and can facilitate the establishment of a stable dominance relationship (Drews, 1993), all while allowing the individuals to remain in the same spatial location after the hostilities cease (Ligon, 2014). In contrast, fleeing creates a physical separation between the competitors and thus may be antithetical to the formation of spatially delimited social groups. When animals are limited in their mobility, it can select for the use of submission displays because of the reduced ability to flee (Mastumura and Hayden, 2006; Ligon, 2014). Restrictions on dispersal unrelated to mobility per se, for example because of habitat saturation, or predation risk may similarly constrain the ability for animals to flee from a conflict. Dispersal into a new territory is a dangerous activity for both *N. pulcher* and *T. temporalis*. The additional burden of establishing social relationships and achieving acceptance within a new social group may make dispersal away from a current territory a particularly daunting challenge in *N. pulcher* (Balshine et al., 2001; Stiver et al., 2005; Hellmann et al., 2015a, 2016). Thus, a group living lifestyle in and of itself incentivizes the use of submissive displays in *N. pulcher* (Heg et al., 2004, Bergmüller et al., 2005, Arnold & Taborksy 2010). Interestingly, large male *N. pulcher* from outside of the group will occasionally challenge the breeder male for his reproductive position (O'Connor et al., 2015a). In these breeder male contests, *N. pulcher* never show submissive behaviour and always flee from their opponent to terminate the contest (O'Connor et al., 2015a). So when there is no social incentive to remain in the same location, *N. pulcher* will flee when losing a fight, similar to *T. temporalis*.

Both *N. pulcher* and *T. temporalis* show very similar repertoires of agonistic displays (Hick et al., 2014). For example, both species, indicate aggressive intention by taking on a head-down posture, while submission is signaled through the opposite pose, with the fish's head up exposing its ventral aspect (Hick et al., 2014; Reddon et al., 2015). The contrasting forms of submissive and aggressive postures in these species appear to conform to Darwin's principle of antithesis, which predicts that signals that are designed to elicit opposite responses from their receivers should evolve towards opposite forms (Darwin, 1872; Hurd et al., 1995). The fact that both *N. pulcher* and *T. temporalis* show a similar submission postures implies that this behaviour was likely present in their common ancestor and thus did not emerge specifically as an adaptation to group living in *N. pulcher*. Group living may have selected for an increased use of this display to deal with frequent and inescapable social conflicts in *N. pulcher,* although additionally or alternatively, these differences between the species may also be partly or wholly due to experience (Arnold and Taborsky, 2010; see below). Submission is a metabolically costly behaviour and apart from maintenance behaviours, is the largest component of the time-energy budget of subordinate *N. pulcher* (Grantner and Taborsky, 1998; Taborsky and Granter, 1998). The greater use of submission by *N. pulcher* than *T. temporalis* suggests an up-regulation in the use of these displays has occurred in *N. pulcher,* either through evolved changes or as result of feedback from social experience. It is possible the establishment of submissive signaling within a species potentiates group living by reducing the costs of frequent social interactions. Therefore the presence of well developed submissive signaling may be an antecedent to the emergence of group living. Studies aimed at testing this hypothesis through experimentation and further comparative work within a phylogenetic framework will be a productive area for future investigation.

Submissive behavior is known to have an important function in promoting hierarchy formation and stabilization in other social species (e.g., Schenkel, 1967; Drews, 1993; Dugatkin, 1997, 2001; Sapolsky, 2005). In the group living *N. pulcher*, submissive behaviour is performed primarily by subordinate individuals and is directed towards those above them in the dominance hierarchy, suggesting that submission displays play a role in the maintenance of the hierarchy in this species (Dey et al., 2013). Our data link different tactics in conflict resolution with the social system, however, the causal relationship remains uncertain. Submissive behavior may be a necessary prerequisite for group living, or appropriate submissive behavior may develop through ontogeny in the group living species as a consequence of frequent social interactions (see Arnold and Taborsky, 2010; Taborsky et al., 2012b; Taborsky and Oliveira 2012). In general, social behaviour is a very flexible trait. It is possible that the differences in social behaviour that we observed could have been caused by different social environments experienced through ontogeny, rather than adaptations to sociality per se. However, we do note that the developmental environment for the fishes in our laboratory studies was similar for both species. Young of both species were raised in single species stock tanks, without predators or established social groups. Fish were held in mixed sex groups of 8 to 12 individuals (approximately equal numbers of males and females) for a minimum of two weeks prior to study in order to minimize species differences due to recent social experience. Further experimental manipulation of the developmental environment may allow these potential relationships to be disentangled, and help to establish the degree to which the species differences we detected are due to evolved differences in social tendencies.

The nonapeptide hormones oxytocin and vasopressin (known as isotocin and vasotocin in teleost fish) are involved in the regulation of social motivation in fish (Thompson and Walton, 2004, 2011; Braida et al., 2011; Reddon et al., 2014), mammals (Lukas et al., 2011; Mooney et al., 2014), and birds (Goodson et al., 2009; Goodson and Kingsbury, 2011; Goodson et al., 2012) and thus these neurohormones may be key proximate substrates of the building blocks of sociality (Goodson, 2013). Recent work in fishes has implicated both of these nonapeptide hormones in the production of submissive behaviour in fish (Godwin and Thompson, 2012). In the mudskipper, *Periophthalmus modestus*, the expression of vasotocin mRNA is greater in the brains of submissively behaving subordinate fish compared to dominant individuals (Kagawa et al., 2013). Similarly, the expression of vasotocin in the parvocellular region of the preoptic area of the hypothalamus is greater in subordinate than in dominant males of the African cichlid *Astatotilapia burtoni*, and greater vasotocin gene expression in this brain area correlates with greater use of submissive behaviour in this species (Greenwood et al., 2008). When *N. pulcher* subordinates housed in naturalistic social groups in the laboratory were given an exogenous administration of isotocin, they increased submissive behaviour (Reddon et al., 2012). This change in behaviour was specific to submission displays, as the treated fish did not show any changes in their aggressive or affiliative behaviour and did not differ compared to control animals. Hellmann et al., (2015b) repeated this experiment on free-living wild fish in Lake Tanganyika and again found that exogenous isotocin increased the expression of submissive behaviour in *N. pulcher*. *Neolamprologus pulcher* have a higher expression of the isotocin gene in their brains than do *T. temporalis* (O'Connor et al., 2015b, 2016). Together, these data suggests that evolution may have acted upon the isotocin system during the divergence of social behaviour in the lamprologines, possibly in part because of its role in promoting submissive behaviour.

In conclusion, in the current study, we identify behaviours that differ between two species of cichlids that diverge in social system, namely, social motivation and conflict resolution behavior. The motivation to approach, interact with, and tolerate other conspecifics is a an essential first step toward social living (Soares et al., 2010; Goodson, 2013), and our results contrasting the group living *N. pulcher* with the non-grouping *T. temporalis* support the hypothesis that the emergence of complex social behaviour has coincided with increased social motivation. Conflict management is another critical aspect of a social phenotype. The greater use of submission displays in the group living *N. pulcher* compared to the non-grouping *T. temporalis* suggests alternation in the conflict management mechanisms during the transition to social living in this group. Social motivation and submissive behaviour are promising candidates for further comparative investigation into how basic behaviors build to form complex social phenotypes. Experimental work that manipulates the expression of these behaviours, and explores the fitness consequences in species that exhibit varying degrees of sociality is a critical next research step.

Acknowledgements

We thank Connie O'Connor for help producing figures and for contributing invaluable comments and discussion during the preparation of this manuscript. We also thank Susan Marsh-Rollo, Sophie St-Cyr, Jennifer Reynolds, Maxwell and John Juma, and Rory and Shefali Nefdt for assistance with field data collection and logistics in Zambia; and Drs. Harris Phiri, Patrick Ngalande, Justina Kasabila, Lawerence Mkasa and Ruben Shapola from the Zambian Department of Fisheries for permission and support for the fieldwork in Lake Tanganyika. We thank Connie O'Connor, Kristina Hick, Susan Marsh-Rollo, Tiffany Tsui and Joanna Tan for assistance with data collection in the laboratory. A Discovery grant from the Natural Sciences Engineering Research Council of Canada and a Canada Foundation for Innovation award to SB supported this research. MW was ssupported by a Canadian Commonwealth Post-doctoral Research Fellowship (DFAIT) and an Ontario Ministry of Research and Innovation (MRI) Post-doctoral Fellowship. AR is currently supported by a Banting Postdoctoral Fellowship.

References

Arnold, C., Taborsky, B., 2010. Social experience in early ontogeny has lasting effects on social skills in cooperatively breeding cichlids**.** Anim. Behav. 79, 621–630. (doi:10.1016/j.anbehav.2009.12.008)

Balshine, S., Leach, B., Neat, F., Reid, H., Taborsky, M., Werner, N., 2001. Correlates of group size in a cooperatively breeding cichlid fish (*Neolamprologus pulcher*). Behav. Ecol. Sociobiol. 50, 134-140.

Balshine-Earn, S., Neat, F.C., Reid, H., Taborsky, M., 1998. Paying to stay or paying to breed? Field evidence for direct benefits of helping behavior in a cooperatively breeding fish. Behav. Ecol. 9, 432-438.

Barlow, G.W., 2002. *The cichlid fishes: nature's grand experiment in evolution*. Basic Books.

Bergmüller, R., Taborsky, M., 2005. Experimental manipulation of helping in a cooperative breeder: helpers 'pay to stay' by pre-emptive appeasement. Anim. Behav. 69, 19-28.

Bergmüller, R., Heg, D., Peer, K., Taborksy, M., 2005. Extended safe havens and between-group dispersal of helpers in a cooperatively breeding cichlid. Behav. 142, 1643-1667.

Brichard, P. 1989. Cichlids and all the other fishes of Lake Tanganyika. THF Publications, Neptune City, NJ.

Braida, D., Donzelli, A., Martucci, R., Capurro, V., Busnelli, M., Chini, B., Sala, M., 2012. Neurohypophyseal hormones manipulation modulate social and anxiety-related behavior in zebrafish. Psychopharmacology, 220, 319-330.

Day, J.J., Santini, S., Garcia-Moreno, J., 2007. Phylogenetic relationships of the Lake Tanganyika cichlid tribe Lamprologini: the story from mitochondrial DNA. Mol. Phylogen. Evol. 45, 629-642.

Darwin, C., 1872. *The Expression of the Emotions in Man and Animals*. London, UK: John Marry

Desjardins, J.K., Hazelden, M.R., Van Der Kraak, G., Balshine, S., 2005. Male and female cooperatively breeding fish provide support for the "challenge hypothesis". Behav. Ecol. 17, 149-154.

Drews, C., 1993. The concept and definition of dominance in animal behaviour. Behaviour, 125, 283-313.

Dey, C.J., Reddon, A.R., O'Connor, C.M., Balshine, S., 2013. Network structure is related to social conflict in a cooperatively breeding fish. Anim. Behav. 85, 395-402.

Dey C.J., O'Connor, C.M., Shultz, S, Balshine, S., Fitzpatrick J., in review. Monogamy and evolutionary transitions to complex societies. Nat. Ecol. Evol.

Desjardins, J.K., Stiver, K.A., Fitzpatrick, J.L., Balshine, S., 2008. "Differential responses to territory intrusions in cooperatively breeding fish". Anim. Behav., 75, 595-604.

Dierkes, P., Heg, D., Taborsky, M., Skubic, E., Achmann, R. 2005. Genetic relatedness in groups is sex-specific and declines with age of helpers in a cooperatively breeding cichlid. Ecol. Lett. 8, 968-975.

Donaldson, Z.R., Young, L.J. 2008. Oxytocin, vasopressin, and the neurogenetics of sociality. Science, 322, 900-904.

Dugatkin, L.A. 1997. Winner and loser effects and the structure of dominance hierarchies. Behav. Ecol. 8, 583-587.

Dugatkin, L.A. 2001. Bystander effects and the structure of dominance hierarchies. Behav. Ecol. 12, 348–352.

Goodson, J.L. 2013. Deconstructing sociality, social evolution and relevant nonapeptide functions. Psychoneuroendocrinology. 38, 465-478.

Godwin, J., Thompson, R., 2012. Nonapeptides and social behavior in fishes. Horm. Behav., 61, 230-238.

Goodson, J.L., Schrock, S.E., Klatt, J.D., Kabelik, D., Kingsbury, M.A. 2009. Mesotocin and nonapeptide receptors promote estrildid flocking behavior. *Science*, *325*(5942), 862-866.

Goodson, J.L., Kingsbury, M.A., 2011. Nonapeptides and the evolution of social group sizes in birds. Frontiers in Neuroantomy doi:10.3389/fnana.2011.00013

Goodson, J.L., Kelly, A.M., Kingsbury, M.A., 2012. Evolving nonapeptide mechanisms of gregariousness and social diversity in birds. Horm. Behav., 61, 239-250.

Goodson, J.L., Evans, A.K., Lindberg, L., Allen, C.D., 2005. Neuro–evolutionary patterning of sociality. Proc. R Soc. Lond. B: Biol. Sci., 272, 227-235.

Grantner, A., Taborsky, M. 1998. The metabolic rates associated with resting, and with the performance of agonistic, submissive and digging behaviours in the cichlid fish *Neolamprologus pulcher* (Pisces: Cichlidae). J Comp Physiol. B, 168, 427-433.

Greenwood, A.K., Wark, A.R., Fernald, R.D., Hofmann, H.A., 2008. Expression of arginine vasotocin in distinct preoptic regions is associated with dominant and subordinate behaviour in an African cichlid fish. Proc. R Soc. Lond. B: Biol. Sci., 275. 2393-2402.

Heg, D., Bachar, Z., Brouwer, L., Taborsky, M. 2004. Predation risk is an ecological constraint for helper dispersal in a cooperatively breeding cichlid. Proc. R Soc. Lond. B: Biol. Sci., 271, 2367-2374.

Heg, D., Bachar, Z. 2006. Cooperative breeding in the Lake Tanganyika cichlid *Julidochromis ornatus*. Environ. Biol. Fish. 76, 265-281.

Heg, D., Bachar, Z., Taborsky, M. 2005. Cooperative breeding and group structure in the Lake Tanganyika cichlid *Neolamprologus savoryi*. Ethology, 111, 1017-1043.

Hellmann, J, Ligocki, I, O'Connor, C, Reddon, A, Garvy, K, Marsh-Rollo, S, Gibbs, L, Balshine, S, and Hamilton, I, 2015a. "Reproductive sharing in relation to group and colony-level attributes in a cooperative breeding fish". Proc. R Soc. Lond. B: Biol. Sci., 282, 20150954

Hellmann, J, Reddon, A, Ligocki, I, O'Connor, C, Garvy, K, Marsh-Rollo, S, Hamilton, I, Balshine, S, 2015b. Changes in submissive behaviour in response to social perturbation: Impacts of isotocin and the social landscape. Anim. Behav., 105, 55-62.

Hellmann, J.K., Sovic M.G., Gibbs H.L., Reddon, A.R., O'Connor C.M., Ligocki I.Y., Marsh-Rollo, S., Balshine S., Hamilton I.M., 2016. Within-group relatedness is correlated with colony-level social structure and reproductive sharing in a social fish. Molecular Ecology. doi: 10.1111/mec.13728

Hick, K., Reddon, A.R., O'Connor, C.M., Balshine, S. 2014. Strategic and tactical fighting decisions in cichlid fishes with divergent social systems. Behavior, 151, 77-71.

Hurd, P.L., Wachtmeister, C.A. Enquist, M., 1995. Darwin's principle of antithesis revisited: a role for perceptual biases in the evolution of intraspecific signals. Proc. R Soc. Lond. B: Biol. Sci., 259, 201-205.

Kagawa, N., Nishiyama, Y., Kato, K., Takahashi, H., Kobayashi, Y., Sakamoto, H., Sakamoto, T., 2013. Potential roles of arginine-vasotocin in the regulation of aggressive behavior in the mudskipper (*Periophthalmus modestus*). Gen. Comp. Endocrinol., 194, 57-263.

Kocher, T. D., 2004. Adaptive evolution and explosive speciation: the cichlid fish model. Nat. Rev. Gen., 5, 288-298.

Konings, A., 1998. Cichlids in their natural habitat. Cichlid Press, El Paso, TX.

Kuwamura, T., 1986. Parental care and mating systems of cichlid fishes in Lake Tanganyika: a preliminary field survey. J. Ethol. 4, 129-146.

Ligon, R.A., 2014. Defeated chameleons darken dynamically during dyadic disputes to decrease danger from dominants. Behav. Ecol. Sociobiol., 68), 1007-1017.

Lukas, M., Toth, I., Reber, S.O., Slattery, D.A., Veenema, A.H., Neumann, I.D., 2011. The neuropeptide oxytocin facilitates pro-social behavior and prevents social avoidance in rats and mice. Neuropsychopharmacology, 36, 2159-2168.

Mboko, S. and Kohda, M. 1999. Piracy mating by large males in a monogamous substrate-breeding cichlid in Lake Tanganyika. J. Ethol. 17, 51-55.

Matsumura, S., Hayden, T.J., 2006. When should signals of submission be given?–A game theory model. J Theor. Biol. 240, 425-433.

Mooney, S.J., Douglas, N.R., Holmes, M.M., 2014. Peripheral administration of oxytocin increases social affiliation in the naked mole-rat (*Heterocephalus glaber*). Horm. Behav., 65, 380-385.

O'Connell, L.A., Hofmann, H.A., 2011. The vertebrate mesolimbic reward system and social behavior network: a comparative synthesis. J Comp. Neurol., 519, 3599-3639.

O’Connor, C.M., Rodela, T.M., Mileva, V.R., Balshine, S., Gilmour, K.M., 2013. Corticosteroid receptor gene expression is related to sex and social behavior in a social fish. Comp. Biochem. Physiol. A, 164, 438-446.

O'Connor, C.M., Reddon, A.R., Ligocki, I.Y., Hellmann, J.K., Garvy, K, Marsh-Rollo, S, Hamilton, I.M., and Balshine, S., 2015a. Motivation but not body size influences territorial contest dynamics in a wild cichlid fish. Anim. Behav., 107, 19-29.

O'Connor, C.M., Marsh-Rollo, S., Cortez Ghio, S., Balshine, S., Aubin-Horth., N., 2015b. Is there convergence in the molecular pathways underlying the repeated evolution of sociality in African cichlids? Horm. Behav., 75, 160-168.

O'Connor, C.M., Marsh-Rollo, S.E., Aubin-Horth, N., Balshine, S., 2016. Species-specific patterns of nonapeptide brain gene expression relative to pair-bonding behaviour in grouping and non-grouping cichlids. Horm. Behav., 80, 30-38.

R Development Core Team. 2012. R: A language and environment for statistical computing. [Online]. Website: http://www.R-project.org/.

Reddon, A.R. and Hurd, P.L., 2009. Differences in aggressive behavior between convict cichlid color morphs: amelanistic convicts lose even with a size advantage. Acta Etholog. 12, 49-53.

Reddon, A.R., O'Connor, C.M., Balshine, S., Gozdowska, M., Kulczykowska, E., 2015. Brain nonapeptide levels are related to social status and affiliative behaviour in a cooperatively breeding cichlid fish. Roy. Soc. Open Sci., 2, 140072.

Reddon, A.R., Voisin, M., O'Connor, C.M., Balshine, S., 2014. Isotocin and sociality in the cooperatively breeding cichlid fish, *Neolamprologus pulcher*. Behaviour, 15, 1389–1411.

Reddon, A.R., O'Connor, C.M., Marsh-Rollo, S.E., and Balshine, S., 2012. Effects of isotocin on social responses in a cooperatively breeding fish. Anim. Behav., 84, 753-760.

Reddon, A.R., Balk, D.B, Balshine, S., 2011a. Sex differences in group-joining decisions in social fish. Anim. Behav. 82, 229-234.

Reddon, A.R., Voisin, M.R., Menon, N., Marsh-Rollo, S.E., Wong, M.Y.L., Balshine, S. 2011b. Rules of engagement for resource contests in a social fish. Anim. Behav. 82, 93-99.

Sapolsky, R.M., 2005. The influence of social hierarchy on primate health. Science, 308, 648-652.

Schenkel, R., 1967. Submission: its features and function in the wolf and dog. Amer. Zool. 7, 319-329.

Soares, M.C., Bshary, R., Fusani, L., Goymann, W., Hau, M., Hirschenhauser, K. and Oliveira, R.F. 2010. Hormonal mechanisms of cooperative behavior. Proc. Roy. Soc. Lon. B, 365, 2737-2750.

Sopinka, N.M., Fitzpatrick, J.L., Desjardins, J.K., Stiver, K.A., Marsh-Rollo, S.E., Balshine, S., 2009. "Liver size reveals social status in the African cichlid Neolamprologus pulcher". J Fish Biol., 75, 1-16.

Stiver, K.A., Dierkes, P., Taborsky, M., Balshine, S., 2004. Dispersal patterns and status change in a co-operatively breeding cichlid *Neolamprologus pulcher*: evidence from microsatellite analyses and behavioural observations. J Fish Biol., 65, 91-105.

Stiver, K.A., Dierkes, P., Taborksy, M., Gibbs, H.L., Balshine, S. 2005. Relatedness and helping in fish: examining the theoretical predictions. Proc. Roy. Soc. Lon. B, 272, 1593-1599.

Sturmbauer, C., Salzburger, W., Duftner, N., Schelly, R., Koblmüller, S., 2010. Evolutionary history of the Lake Tanganyika cichlid tribe Lamprologini (Teleostei: Perciformes) derived from mitochondrial and nuclear DNA. Mol. Phylogen. Evol., 57, 266-284.

Taborsky, M., 1984. Broodcare helpers in the cichlid fish *Lamprologus brichardi*: their costs and benefits. Anim. Behav. 32, 1236–1252.

Taborsky, M., 1994. Sneakers, satellites, and helpers: parasitic and cooperative behavior in fish reproduction. Adv. Study Behav., 23, 1-100.

Taborsky, M., Limberger, D., 1981. Helpers in fish. Behav. Ecol. Sociobiol. 8, 143-145.

Taborsky M., Grantner, A., 1998. Behavioural time–energy budgets of cooperatively breeding, *Neolamprologus pulcher* (Pisces: Cichlidae). Anim. Behav., 56, 1375-1382.

Taborsky, B., Oliveira, R.F., 2012. Social competence: an evolutionary approach. Trends Ecol. Evol. 27, 679-688.

Taborsky, B., Arnold, C., Junker, J. and Tschopp, A., 2012. The early social environment affects social competence in a cooperative breeder. Anim. Behav. 82, 1067-1074.

Taves, M.D., Desjardins, J.K., Mishra, S. and Balshine, S., 2009. Androgens and dominance: sex-specific patterns in a highly social fish (*Neolamprologus pulcher*). Gen. Comp. Endocrinol. 161, 202-207.

Thompson, R. R., Walton, J. C., 2004. Peptide effects on social behavior: effects of vasotocin and isotocin on social approach behavior in male goldfish (*Carassius auratus*). Behav. Neurosci. 118, 620-626.

Wong, M.Y.L., Balshine, S. 2011. The evolution of cooperative breeding in the African cichlid fish, *Neolamprologus pulcher*. Biol. Rev. 86, 511-530.

Zayed, A., Robinson, G.E., 2012. Understanding the relationship between brain gene expression and social behavior: lessons from the honey bee. Ann. Rev. Gen., 46, 591-615.

Figure captions

Figure 1. (A) *Telmatochromis temporalis* and (B) *Neolamprologus pulcher* are two closely related Lamprologine cichlid fishes that are similar in body size, appearance, and ecology, but differ in social system. *Neolamprologus pulcher* are group living while *T. temporalis* non-grouping. Photo credits: Susan Marsh Rollo and Jen Reynolds.

Figure 2. Experimental setup for the social motivation assay. (A) During a 10 min acclimation period, the focal fish was confined within a transparent cylinder. (B) Following acclimation, the transparent cylinder was lifted, and the focal fish was allowed to interact with the stimulus fish across the barrier of the transparent cylinder or enter the shelter over a 15 min trial duration.

Figure 3. Experimental setup for the conflict assay. (A) During a 1 hr acclimation period, the two contestants were each given a shelter, but were separated from each other by opaque dividers. (B) Following acclimation, the outer shelters and barriers were removed, and fish were allowed to compete over the remaining central shelter for 30 min.

Figure 4. Social motivation measured in wild breeding individuals of a group-living cichlid (*Neolamprologus* pulcher) and a non-grouping cichlid (*Telmatochromis temporalis*). (A) Compared to the *T. temporalis*, *N. pulcher* displayed overall more social behaviors (i.e., affiliative, submissive, and aggressive displays) and (B) social behavior constituted a higher proportion of all observed behaviors. (C) After controlling for group size, there was no difference in the number of social behaviours produced per group member.

Figure 5. In a controlled laboratory test of social motivation, (A) both species were equally likely to visit the fish or the shelter stimulus first, and (B) there was no species difference in the time spent with

the stimulus fish, however, (C) *N. pulcher* spent more time interacting with a conspecific than did *T. temporalis*.

Figure 6. Conflict resolution behavior measured in a group-living cichlid (*Neolamprologus* pulcher) and a non-grouping cichlid (*Telmatochromis temporalis*). During staged contests in the laboratory, *N. pulcher* displayed (A) less aggression, were (B) more likely to use submissive displays, and were (C) less likely than *T. temporalis* to flee from their opponent. At the individual level, (D) members of both species that produced high rates of submission rarely fled from their opponents.

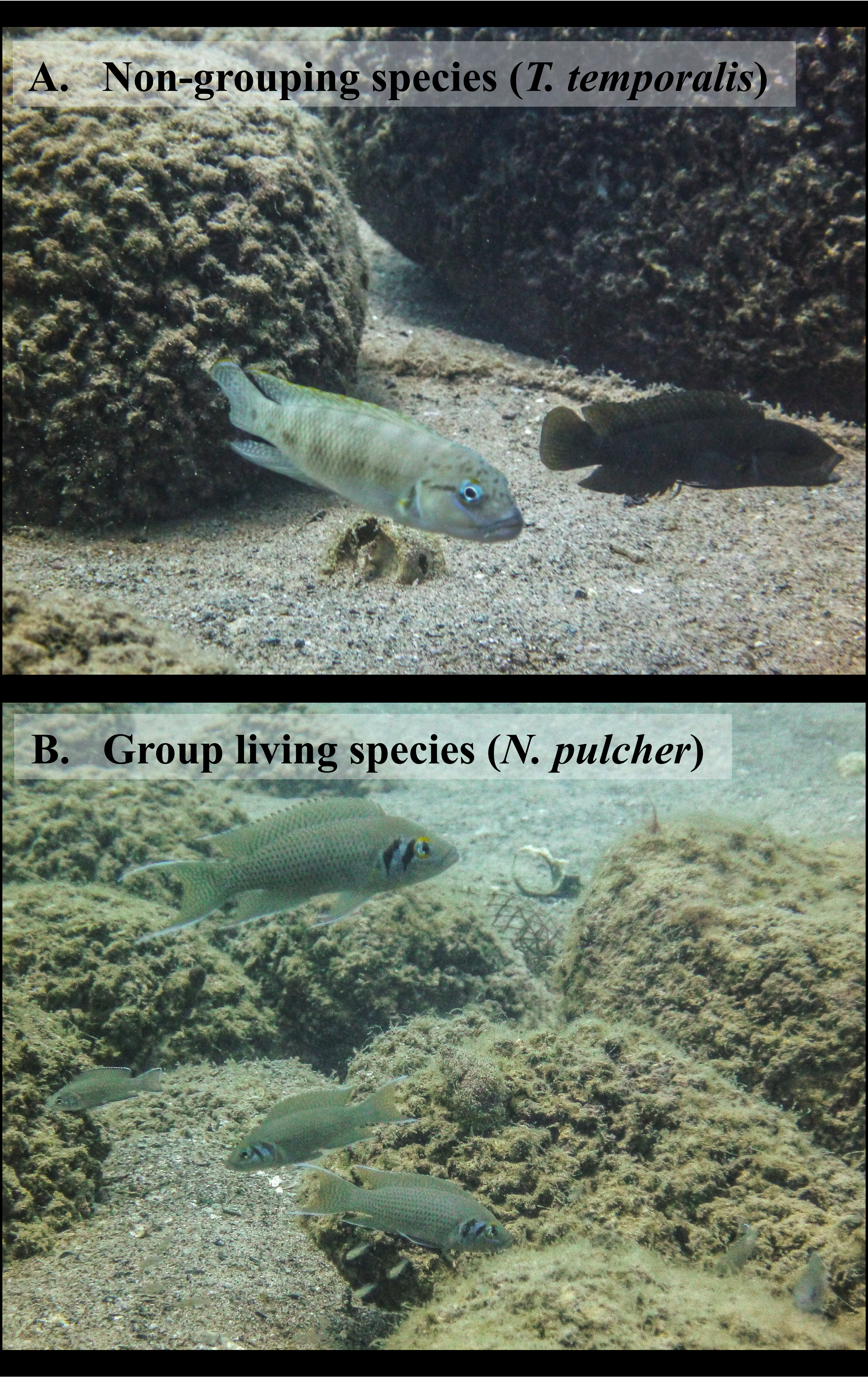
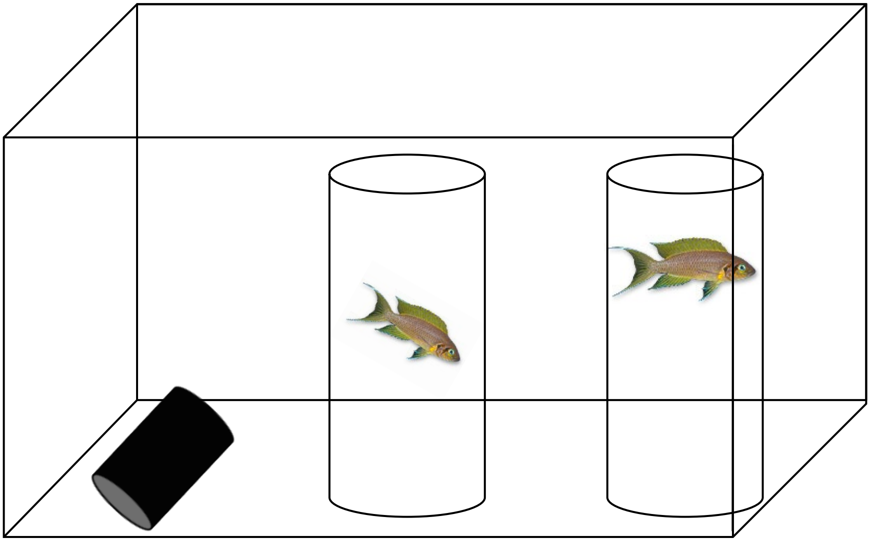

A. Non-grouping species (*T. temporalis*)
B. Group living species (*N. pulcher*)

Figure 2

A. 10 min acclimation



B. 15 min trial

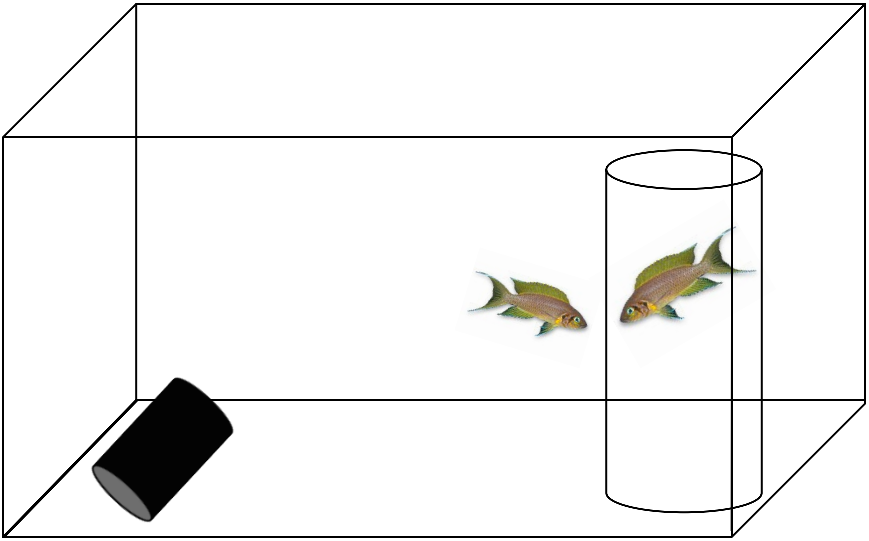
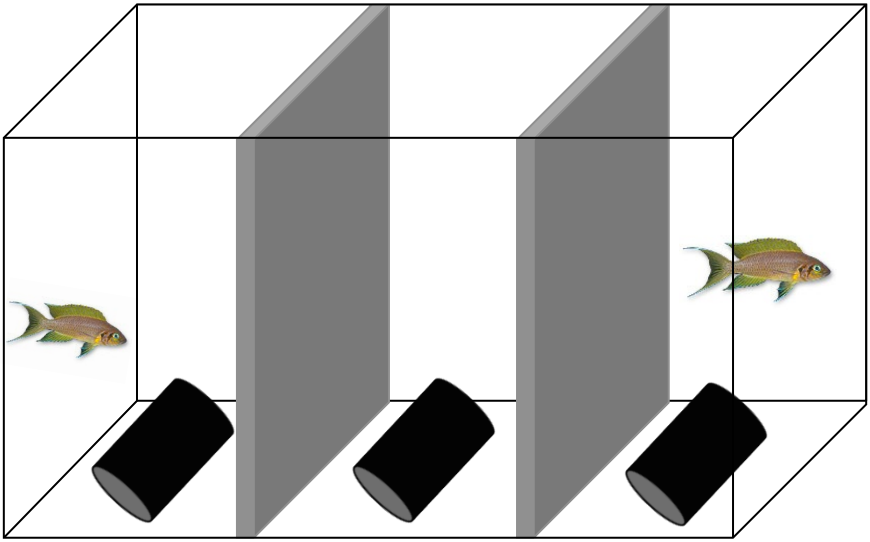


Figure 3

A. 60 min acclimation



B. 30 min trial

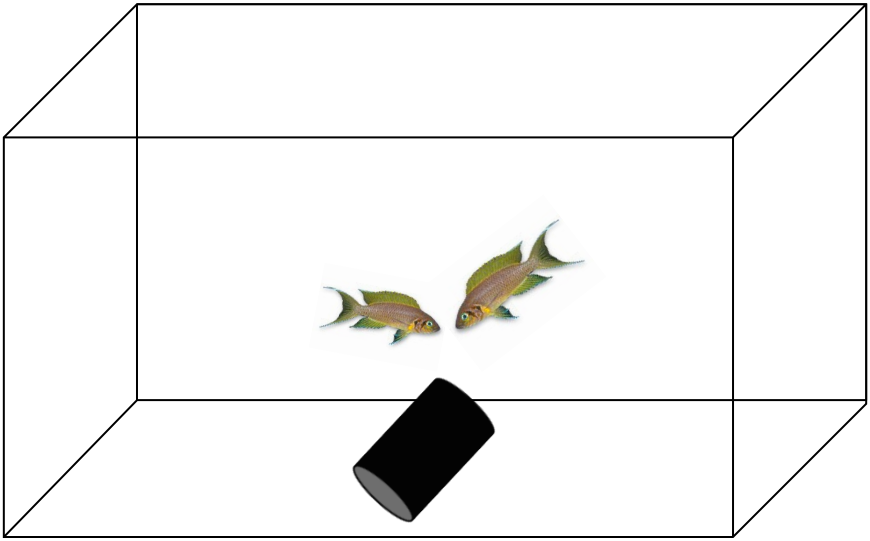


Figure 4

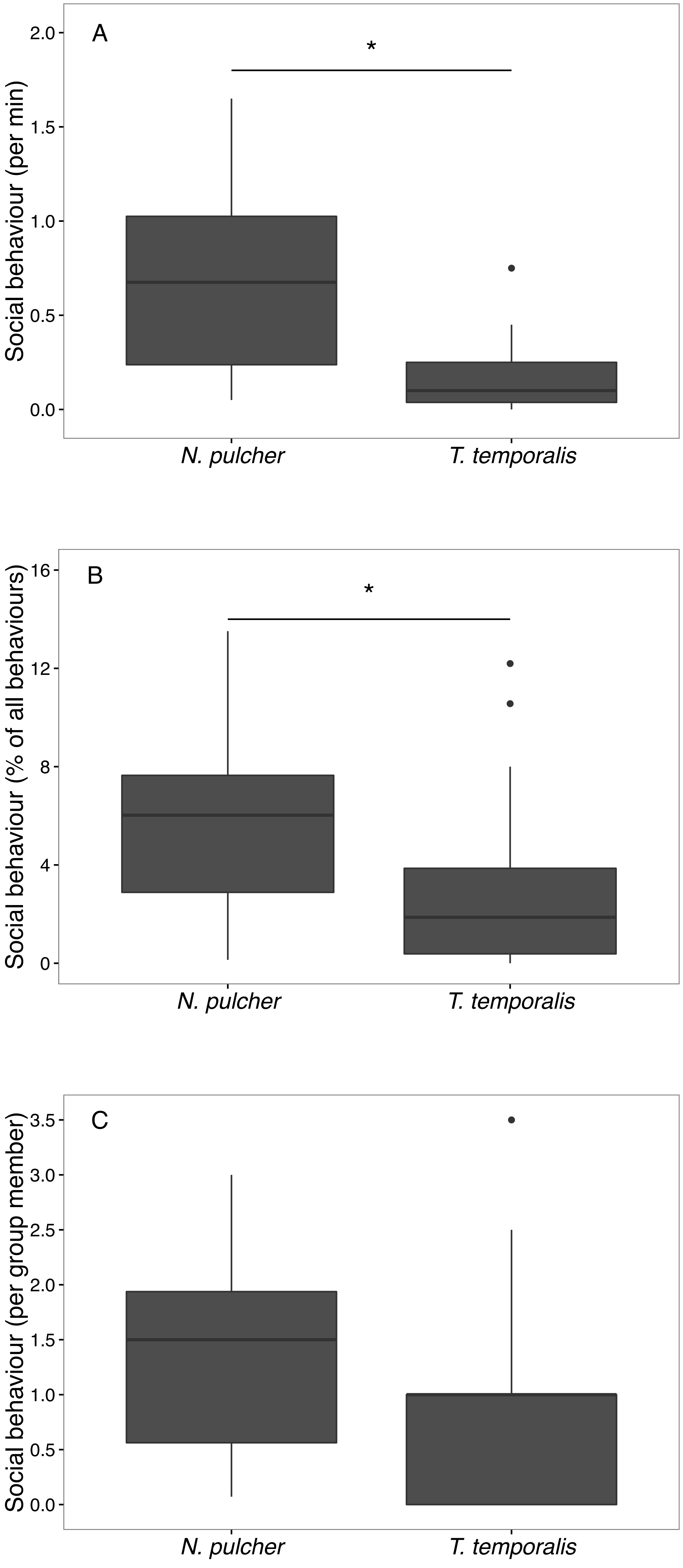

A
*
Social behaviour (per min)
2.0
1.5
1.0
0.5
0.0
N. pulcher
T. temporalis
B
*
Social behaviour (% of all behaviours)
16
12
8
4
0
N. pulcher
T. temporalis
C
Social behaviour (per group member)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
N. pulcher
T. temporalis

Figure 5

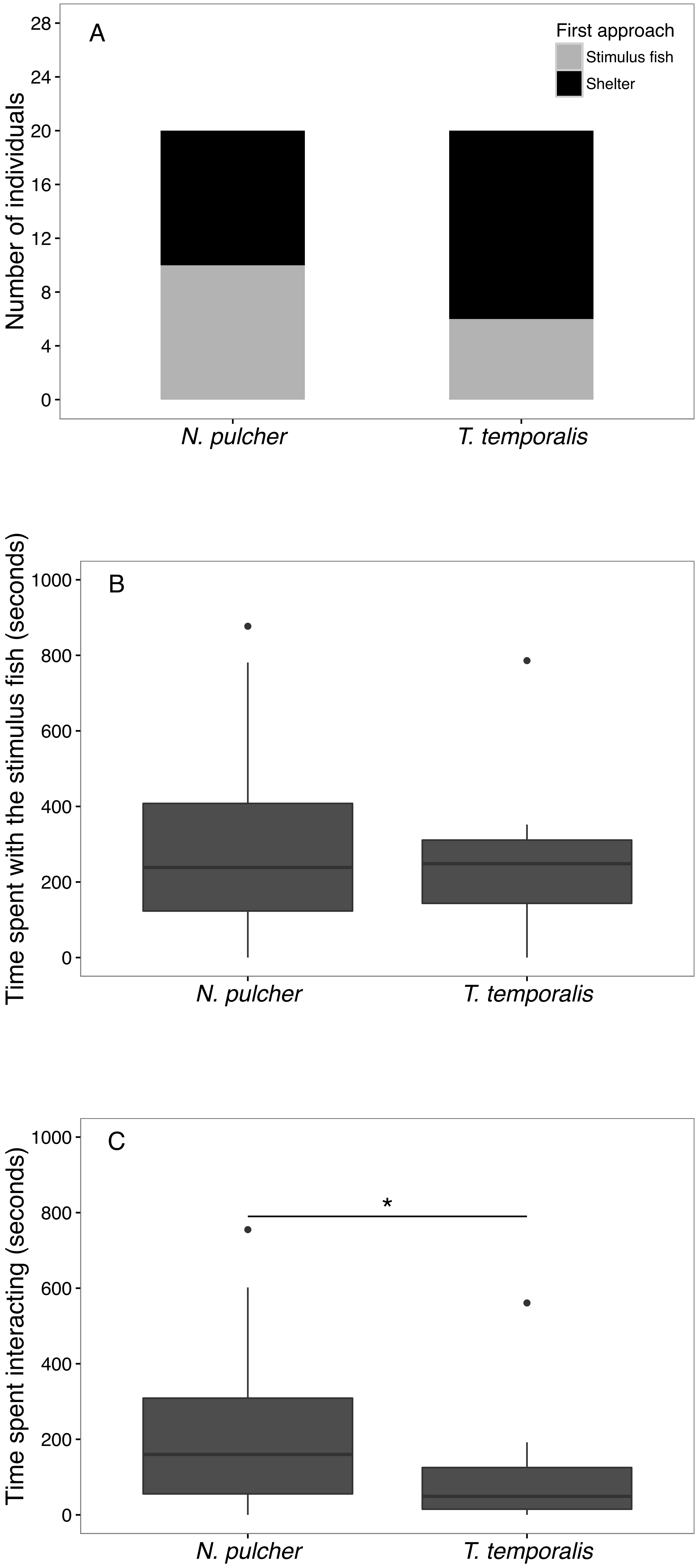
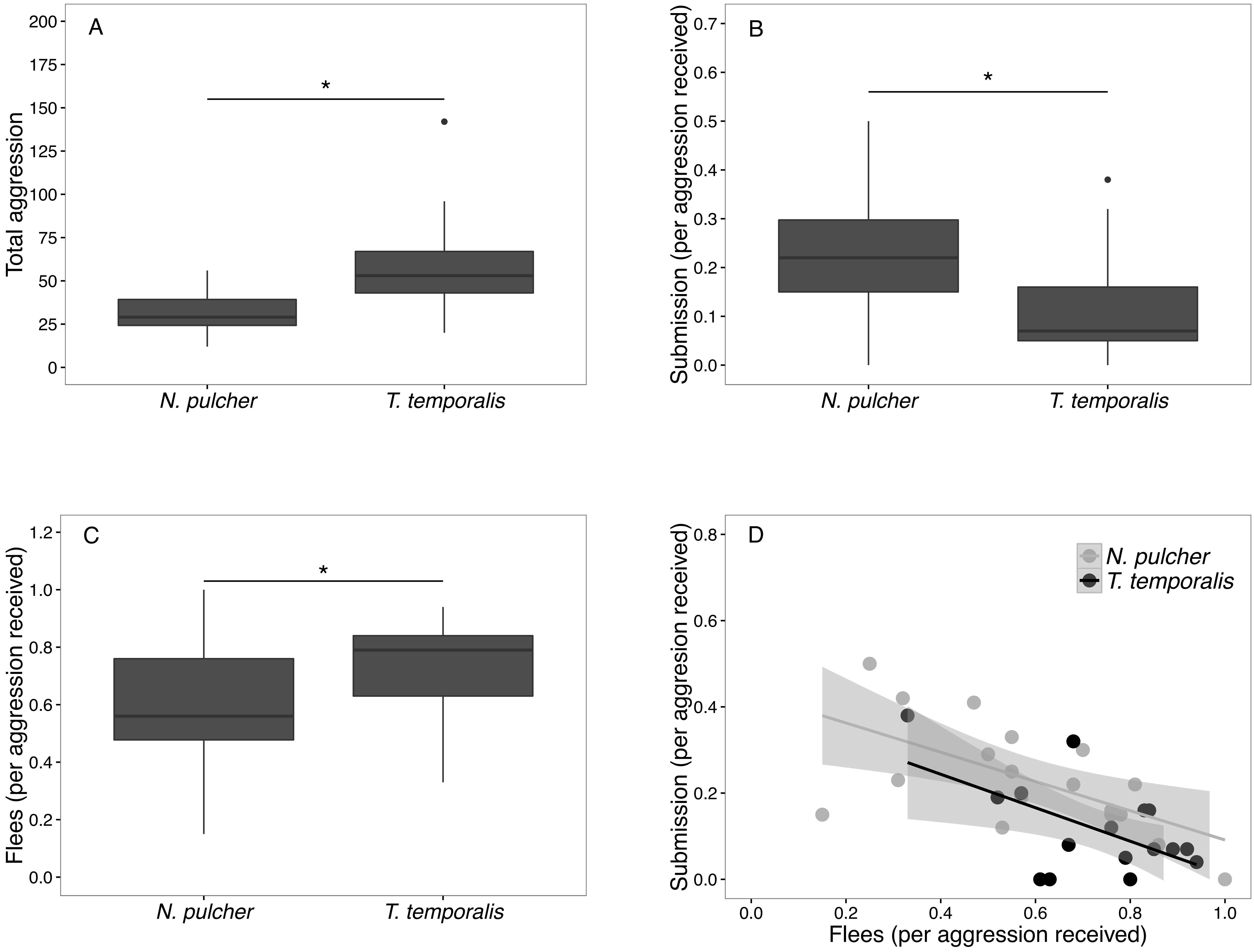

A
First approach
Stimulus fish
Shelter
Number of individuals
0
4
8
12
16
20
24
28
N. pulcher
T. temporalis
B
Time spent with the stimulus fish (seconds)
0
200
400
600
800
1000
N. pulcher
T. temporalis
C
Time spent interacting (seconds)
*
0
200
400
600
800
1000
N. pulcher
T. temporalis

Figure 6


A
Total aggression
0
25
50
75
100
125
150
175
200
*
N. pulcher
T. temporalis
B
Submission (per aggression received)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
*
N. pulcher
T. temporalis
C
Flees (per aggression received)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
*
N. pulcher
T. temporalis
D
Submission (per aggresion received)
0.0
0.2
0.4
0.6
0.8
Flees (per aggression received)
0.0
0.2
0.4
0.6
0.8
1.0
N. pulcher
T. temporalis