Animal Behaviour 105 (2015) 55–62

Contents lists available at ScienceDirect

Animal Behaviour

journal homepage: www.elsevier.com/locate/anbehav

Group response to social perturbation: impacts of isotocin and the social landscape

Jennifer K. Hellmann [a, †], Adam R. Reddon [b, *, †], Isaac Y. Ligocki [a],
Constance M. O'Connor [b], Kelly A. Garvy [c], Susan E. Marsh-Rollo [b], Ian M. Hamilton [a, d],
Sigal Balshine [b]

[a] *Department of Evolution, Ecology, and Organismal Biology, The Ohio State University, Columbus, OH, U.S.A.*
[b] *Aquatic Behavioural Ecology Lab, Department of Psychology, Neuroscience, and Behaviour, McMaster University, Hamilton, ON, Canada*
[c] *Department of Environmental Science & Policy, Duke University, Durham, NC, U.S.A.*
[d] *Department of Mathematics, The Ohio State University, Columbus, OH, U.S.A.*

ARTICLE INFO

Article history:
Received 8 November 2014
Initial acceptance 12 January 2015
Final acceptance 5 March 2015
Published online
MS. number: A14-00907R

Keywords:
biological market theory
cooperative breeding
neighbour
Neolamprologus pulcher
nonapeptide
oxytocin
submissive behaviour

Conflict is an inherent part of group living, and the mediation of conflict is essential for the stability of social groups. Response to within-group social conflict should depend on the external social environment. Individuals in dense social neighbourhoods have greater opportunities to disperse and join a nearby group compared to individuals in sparse social neighbourhoods with few nearby groups. To explore the influence of the social neighbourhood on responses to conflict, we experimentally perturbed groups of wild *Neolamprologus pulcher*, a cooperatively breeding cichlid fish, by temporarily removing a subordinate individual. Such removals typically increase the amount of within-group aggression. As predicted, aggression towards the returning subordinate and the rate of eviction from the group increased with the density of neighbouring social groups. Furthermore, we predicted that the returning subordinate could improve its likelihood of reacceptance into the group by displaying submissively. To test this prediction, we attempted to manipulate submissive behaviour by injecting the removed individuals with isotocin, a nonapeptide hormone that has been shown in the laboratory to increase the expression of submissive behaviour in this species. As predicted, subordinates that received isotocin showed more submission when returned to their group. However, contrary to our prediction, these isotocin-treated fish received more aggression from their group-mates and were more likely to be evicted than fish receiving a saline control injection. Our results emphasize the importance of the social neighbourhood in determining within-group dynamics but surprisingly contradict the notion that submissive behaviour reduces aggression and facilitates group stability.

Conflict is an unavoidable consequence of group living because individuals seek to maximize their share of resources and reproductive opportunities within the group, thereby reducing the resources and reproduction available for others. Conflict among individuals within a social group can counteract the benefits of group living, leading to reduced group productivity, injury, eviction and group dissolution (Aureli, Cords, & van Schaik, 2002). Therefore, in group-living species there should be strong selection for behaviours that mitigate conflict and reduce disputes within the group (Aureli et al., 2002; Bourke, 2011; Cant & Johnstone, 2009; Thompson, Donaldson, Johnstone, Field, & Cant, 2014).

Subordinate individuals often make use of submissive displays to appease dominant group members and increase their likelihood of being tolerated within the group (Bergmüller & Taborsky, 2005; Huntingford & Turner, 1987; Wilson, 1975). Subordinate individuals may perform submissive displays as an explicit signal of deference to dominant individuals in order to pre-empt or terminate a conflict. Submissive displays allow the signaller to concede a conflicted resourse without the need for further costly aggression (Lorenz, 1966; Matsumura & Hayden, 2006). They also allow the signaller to remain in the same spatial location rather than fleeing from the dominant (Issa & Edwards, 2006; Ligon, 2014; Matsumura & Hayden, 2006). Consequently, the expression of

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* Correspondence and present address: A. R. Reddon, Department of Biology, McGill University, 845 Sherbrook Street West, Montreal, QC H3A 0G4, Canada.
E-mail address: adam.reddon@mcgill.ca (A. R. Reddon).
† Authors made equal contributions.

http://dx.doi.org/10.1016/j.anbehav.2015.03.029
0003-3472/

submissive behaviour is essential for the formation and maintenance of stable groups (Hick, Reddon, O'Connor, & Balshine, 2014; Schenkel, 1967).

The use of submissive displays may not be consistent across all social contexts, as the social environment may alter the amount of within-group conflict as well as the fitness consequences of unresolved conflict (Clutton-Brock, Hodge, & Flower, 2008; Kutsukake & Clutton-Brock, 2008a). For example, individual subordinate members may be more expendable in larger social groups than in smaller social groups (biological market theory; Kutsukake & Clutton-Brock, 2008a; Noë & Hammerstein, 1994). Similarly, groups living in densely populated areas may have a greater ability to attract new members from neighbouring groups, which may decrease the importance of current members and consequently, decrease dominant tolerance of subordinate behaviour (Noë & Hammerstein, 1994). Therefore, regardless of a subordinate's effort to moderate conflict, dominants may not reduce their policing or punishment of subordinates when there is a low cost to losing current subordinates. The social landscape can also alter a subordinate's willingness to avoid within-group conflict. An increased number of neighbouring groups can facilitate successful dispersal to another group (Bergmüller, Heg, Peer, & Taborsky, 2005; Drewe, Madden, & Pearce, 2009; Heg, Heg-Bachar, Brouwer, & Taborsky, 2008) and, therefore, in areas with many groups nearby, subordinates may have a decreased incentive to expend energy on mitigating conflict within their current group (Bergmüller, Heg, & Taborsky, 2005; Zöttl, Chapuis, Freiburghaus, & Taborsky, 2013).

In this study, we sought to understand how the external social environment, the expression of submissive behaviour and the interaction between these factors affect group member responses to within-group conflict. We experimentally perturbed groups of wild *Neolamprologus pulcher*, a cooperatively breeding cichlid fish, by temporarily removing a subordinate group member and manipulating submissive behaviour by administering the nonapeptide hormone isotocin before returning the removed subordinate. *Neolamprologus pulcher* are endemic to Lake Tanganyika, East Africa where they live clustered in colonies composed of 2–200 distinct social groups (Heg, Brouwer, Bachar, & Taborsky, 2005; Stiver et al., 2007). Each social group consists of a dominant breeding pair and 1–15 subordinates (Wong & Balshine, 2011a) that jointly defend permanent territories. Subordinates form size-based dominance hierarchies and care for the offspring of the dominant breeders (Taborsky & Limberger, 1981) until reaching breeding status by inheriting their current territory (Balshine-Earn, Neat, Reid, & Taborsky, 1998; Dierkes, Heg, Taborsky, Skubic, & Achmann, 2005; Wong & Balshine, 2011a) or dispersing to fill vacant breeding positions in other territories (Bergmüller, Heg, Peer, et al., 2005; Stiver et al., 2007). Subordinate *N. pulcher* spend the majority of their time in their own territory, but also visit nearby groups (preferentially visiting groups within a 3 m radius; Heg et al., 2008) and receive little aggression when visiting these nearby groups (Bergmüller, Heg, Peer, et al., 2005). Subordinate removal treatments in *N. pulcher* simulate a dereliction of cooperative duties (Wong & Balshine, 2011a). Such removals also induce rank conflict among the remaining group members as they jockey for position in the perturbed hierarchy (Wong & Balshine, 2011b). Removals can result in punishment from the other group members, including eviction from the group (Balshine-Earn et al., 1998; Fischer, Zöttl, Groenewoud, & Taborsky, 2014). In the current study, we removed a subordinate fish for ~4 h. We predicted that removing subordinates would increase the amount of aggression they received from other group members and would increase the amount of submission given by the removed subordinate. Control fish were removed only briefly (~5 min) to account for potential effects of capture and handling, and to allow administration of the hormone treatment (see below).

Submissive signals are well developed in *N. pulcher* (Bender et al., 2006; Bergmüller & Taborsky, 2005; Bruintjes & Taborsky, 2008; Dey, Reddon, O'Connor, & Balshine, 2013; Reddon, O'Connor, Marsh-Rollo, & Balshine, 2012; Reddon et al., 2015; Reddon et al., 2011; Taborsky, 1985) and appear to facilitate social stability in this species (Hick et al., 2014). To explicitly examine the role of submissive behaviour in modulating the group's response to the focal subordinate, we gave each focal subordinate an injection of isotocin (IT), the teleost fish homologue of oxytocin (Godwin & Thompson, 2012; Thompson & Walton, 2013), or a saline vehicle control, before returning it to the group. In a previous study using a similar design in captive *N. pulcher* groups, we found that IT-treated fish increased their submissive behaviour upon return to their group, but did not show any change in aggressive or affiliative behaviours (Reddon et al., 2012). We predicted that individuals who received an injection of IT in the field would act more submissively, and that this submission would appease dominant group members, reducing the likelihood of these removed individuals being evicted from the group (Bergmüller & Taborsky, 2005).

We also expected that the social context would be an important predictor of both the focal fish's response and its group's response to the social conflict induced by the removal treatment. Because submissive behaviours are costly (Grantner & Taborsky, 1998), we would expect subordinates to scale their use to the potential cost of eviction. Therefore, submissive behaviours should be more valuable in low-density areas where individuals have a reduced ability to disperse to neighbouring groups (Bergmüller, Heg, & Taborsky, 2005; Cant & Johnstone, 2009). We also predicted that returned subordinates would receive more aggression from dominants and suffer increased rates of eviction in denser social neighbourhoods and in larger groups, due to the relative expendability of current subordinates when there are many subordinates in the group and/or a larger pool of potential subordinates that could join the group (Kutsukake & Clutton-Brock, 2008a; Noë & Hammerstein, 1994).

METHODS

We observed 40 *N. pulcher* groups from February to April 2013 in Kasakalawe Bay, Zambia (8°46′S, 31°46′E) using SCUBA. Our experimental groups were clustered in a single colony at a depth of 10–12 m. We mapped and measured the distances between each group in the colony using a 50 m measuring tape. We recorded the size of each experimental group and identified the dominant and subordinate fish in each group. We only considered individuals >10 mm in standard length (SL) in our calculation of group size, and any eggs, larvae or small juveniles that may have been present were not counted. Individuals were considered to be part of the focal group if they were in the territory and swam repeatedly under the rocks without eliciting aggression from other fish within the territory. We selected the largest subordinate in each experimental group as the focal fish for treatment in our experiment. All focal fish used were sexually mature (>35 mm SL; mean SL ± SE: 40.3 ± 0.66 mm; Taborsky, 1985). Between 0900 and 1700 hours, we observed each of these 40 focal subordinate fish for 10 min prior to capture and recorded all social behaviours produced and received. Following published ethograms for this species (Hick et al., 2014; Reddon et al., 2015; Sopinka et al., 2009), we categorized all social behaviours as overt physical attacks (ram, bite, mouth fight), restrained aggressive displays (operculum spreads, fin raises, head shakes), submissive displays (tail quivers, hook displays, submissive postures) or affiliative behaviours (parallel swims, follows, soft touches). We also recorded any aggression produced by the focal fish towards any heterospecific fish. Finally, we recorded the amount of time each focal individual spent hiding under rocks or in shelters within the experimental group's territory during the observation period.

We then captured, measured and sexed each focal fish (23 males, 17 females) before marking each with a dorsal fin clip for visual identification. We returned 20 of the focal fish to their group 5 min later (removal control) while the remaining 20 fish were held away from their group for approximately 4 h (removal treatment; range 3.5–5.5 h; the specific removal duration did not significantly influence any of the results reported below). Focal fish were randomly assigned to either the removal treatment or control condition. Neither group size, nor the density of neighbouring groups significantly differed between fish assigned to the removal or control treatments. Removed fish were held inside an opaque minnow bucket (6 litre) with a number of small holes that allowed for water circulation. The bucket was placed at least 5 m away from the social group of each focal fish. The focal fish were unable to see or interact with any other fish during that time and did not appear to be stressed when released.

Before being returned to their social group, we injected all fish with either isotocin (1 μg/g body mass; 19 fish) dissolved in 0.9% saline solution or with a 0.9% saline control (21 fish). We selected this dose of IT because it has previously been shown to increase submissive behaviour in N. pulcher in the laboratory (Reddon et al., 2012). Neither group size nor the density of neighbouring groups differed significantly between the control and IT-treated fish. A trained experimenter, blind to the treatment conditions, administered the injections and conducted the behavioural observations. We standardized the injection volume by estimated body mass (10 μl/g). Because fish cannot be accurately weighed underwater, we used SL to estimate body mass. We used the tight correlation between body length and body mass ($r = 0.97$; based on 63 *N. pulcher* captured from the same field site during the same field season for another unrelated study; Hellmann et al., 2015) to estimate fish mass. We injected the focal fish between the pelvic fin and anus into the peritoneal cavity using a 0.3 cc insulin syringe with a 31-gauge needle. The needles were guided anteriorly against the body of the fish and penetrated approximately 1–2 mm into the peritoneum before injecting the solution. We gave the focal fish 5 min to recover from handling and injection before being released back into their territory. We allowed the focal fish another 5 min to acclimate to their release prior to beginning a second 10 min behavioural observation in which we collected all of the same behavioural measures as in the pre-treatment observation. If the focal subordinate was driven away from the territory during this second observation period, we considered this an eviction from the group.

Statistical Analysis

We used Mann–Whitney *U* tests to determine whether there were differences in pre- versus post-removal behaviour for both the control and removal treatments. As expected, in the control trials, there were no differences between pre- and post-treatment levels of aggression received from the group or of submission given by the subordinate (see Supplementary Table S1); therefore we analysed only the removal treatments for these measures. We did however find a difference between pre- and post-treatment measures of the time that the focal fish spent hiding in the control trials, suggesting that handling and capture had an effect on hiding behaviour (see Supplementary Table S1). Therefore, for hiding behaviour, we examined both control and removal treatments. For this model, we included a fixed effect of removal treatment to account for variation in behaviour due to this treatment.

Predictors of group aggression directed towards the returned subordinate (pooled counts of overt aggression and restrained aggressive displays) were tested using generalized linear mixed models (GLMM) with a Poisson distribution and a log link function (R version 3.0.2, package lme4). To explore variation in submission given by the focal subordinate towards its group members, we used generalized linear mixed models (GLMM) with a negative binomial distribution (R version 3.0.2, package glmmADMB) because the submission count data were overdispersed. We also used linear mixed effects models to test predictors of the amount of time a subordinate spent hiding under rocks in its territory once released and generalized linear models with binomial error distribution to test the binary response variable of rates of acceptance/eviction at the time of return.

Fixed effects of density (mean ± SD number of neighbouring groups within a 3 m radius of the focal group, a continuous factor: 10.1 ± 6.3), group size (a continuous variable, mean ± SD: 7.6 ± 2.9), hormone treatment (IT versus saline, a categorical variable), sex (male versus female, a categorical variable), and pre-removal behaviour (a continuous factor) were entered into all models. Observer identity was also included as a random effect. Model selection was optimized based on AIC values and log-likelihood tests.

Ethical Statement

We marked the focal fish by removing a small amount of tissue from the dorsal fin. The fish recover from this procedure quickly (Stiver, Dierkes, & Taborsky, 2004) and the removed fin tissue regrows within a week or two. The IT and saline injections did not appear to cause distress in the fish, consistent with previous work on captive *N. pulcher* (Reddon et al., 2012; Reddon, Voisin, O'Connor, & Balshine, 2014). The effects of exogenous IT injections are short lived in *N. pulcher* (Reddon et al., 2014). All aggressive behaviour observed in this study was within the range of normal behaviour observed among unmanipulated individuals in the wild and no fish sustained any visible injuries during our observations. *Neolamprologus pulcher* is not an endangered or a threatened species, and no fish were sacrificed as part of this experiment. The Zambian Department of Fisheries, The Ohio State University chapter of IACUC (protocol ID 2008A0095) and the Animal Research Ethics Board of McMaster University (Animal Utilisation Protocol Number 14-02-05) approved all methods used in this experiment. Our procedures adhered to the guidelines of the Canadian Council for Animal Care, and the Animal Behavior Society/Association for the Study of Animal Behaviour.

RESULTS

Isotocin Treatment

Focal subordinates that received IT produced more frequent submissive displays than those that received the control saline injection treatment (Table 1, Fig. 1). Following previous work (Hick et al., 2014; O'Connor et al., 2013; Reddon et al., 2012), we also measured submission per aggressive act received and found that submission per aggressive act received was higher in isotocin-treated fish than in the saline-treated control fish (GLME: $Z_{16} = 2.00$, $P = 0.045$). Focal subordinates that received IT were subject to more aggression from their group-mates upon their return (Table 1, Fig. 2a) and were more likely to be evicted from their groups (Table 1, Fig. 3a) compared with fish treated with saline.

The Influence of the Social Environment

Subordinates from groups in denser areas of the colony received more aggression upon return to the group compared to subordinates from groups in less dense areas (Table 1, Fig. 2b).

Table 1

Best-fit models illustrating effects on aggression received from the group (GLMM), submission given by the focal subordinate (GLMM), time spent hiding post-return (LMM) and rates of subordinate eviction/re-acceptance into the group (logistic regression)

Predictors	Aggression received		Submission given		Time spent hiding		Eviction/re-acceptance	
	Z_{13}	P	Z_{15}	P	Z_{30}	P	Z_{14}	P
Pre-removal behaviour	−2.00	**0.046**	−1.57	0.12	−0.87	0.39	—	—|
IT treatment[a]	3.69	**<0.001**	3.55	**<0.001**	−3.52	**0.001**	2.05	**0.048**
Density[b]	2.72	**0.006**	—	—	−2.50	**0.018**	1.98	**0.041**
Group size	2.01	**0.044**	—	—	−2.69	**0.012**	—	—
Sex	—	—	—	—	0.52	0.61	—	—
Treatment∗density	—	—	—	—	3.26	**0.003**	—	—

Dashes refer to factors that were not included in the best-fit models. Significant P values ($\alpha < 0.05$) are shown in bold.

[a] Isotocin.

[b] Neighbouring groups within a 3 m radius of the focal group.

Similarly, the probability of eviction from the group increased as the density of surrounding groups increased (Table 1, Fig. 3b). There was no effect of group size on the likelihood of eviction, but the amount of aggression the returning subordinate received from the other group members significantly increased as group size increased (Table 1). Neither group size nor the density of neighbouring groups was a significant predictor of the rate of focal subordinate submission upon return to the group (Table 1).

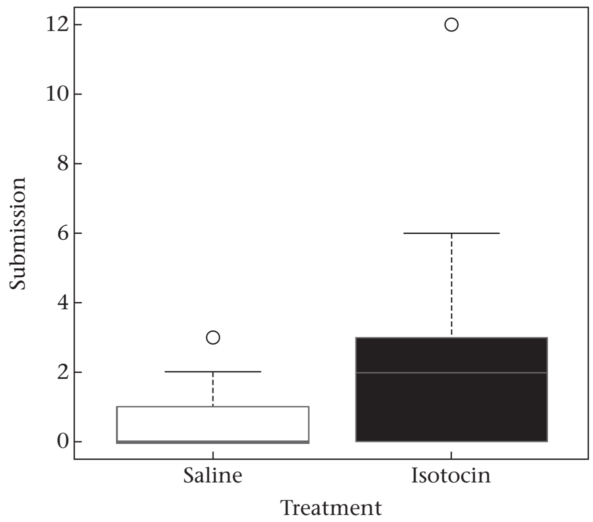

Figure 1. Submission given by subordinates in the isotocin treatment and in the saline control treatment. Box plots show the median, first and third quartile, and the 95% confidence intervals (whiskers).

Interaction between IT Treatment and the Social Environment

Focal subordinates rarely hid underneath the territory rocks before their removal from the group (mean ± SE time spent hiding: 15 ± 4 s), but often hid after being returned to the group (mean ± SE: 226 ± 36 s). There was no difference in the amount of time spent hiding between control and removal treatments ($t_{30} = -0.68$, $P = 0.50$). Post-return hiding time was significantly predicted by an interaction between IT treatment and the density of surrounding groups. Individuals that received saline spent less time hiding as the density of neighbouring groups increased (Fig. 4a) whereas individuals that received IT spent more time hiding as the density of neighbouring groups increased (Fig. 4b).

The amount of time spent hiding was not significantly correlated with the amount of aggression the subordinate received from members of its group (GLMM: removal: $Z_{12} = -0.84$, $P = 0.40$; control: $Z_{12} = -1.08$, $P = 0.28$). There was no correlation between the amount of hiding and the amount of submission in removal control treatments (GLMM: $Z_{16} = -0.21$, $P = 0.84$); however, in removal trials, subordinate submission tended to decrease as the amount of hiding increased (GLMM: $Z_{16} = -1.85$, $P = 0.06$).

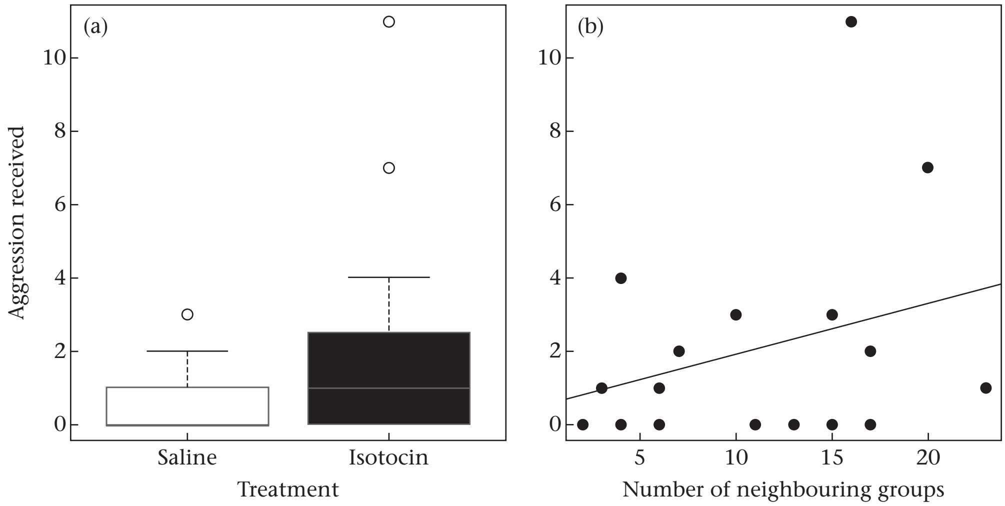

Figure 2. Aggression given by the group towards the returned subordinate plotted against (a) hormone treatment and (b) density of surrounding groups. Box plots show the median, first and third quartile, and the 95% confidence intervals (whiskers).

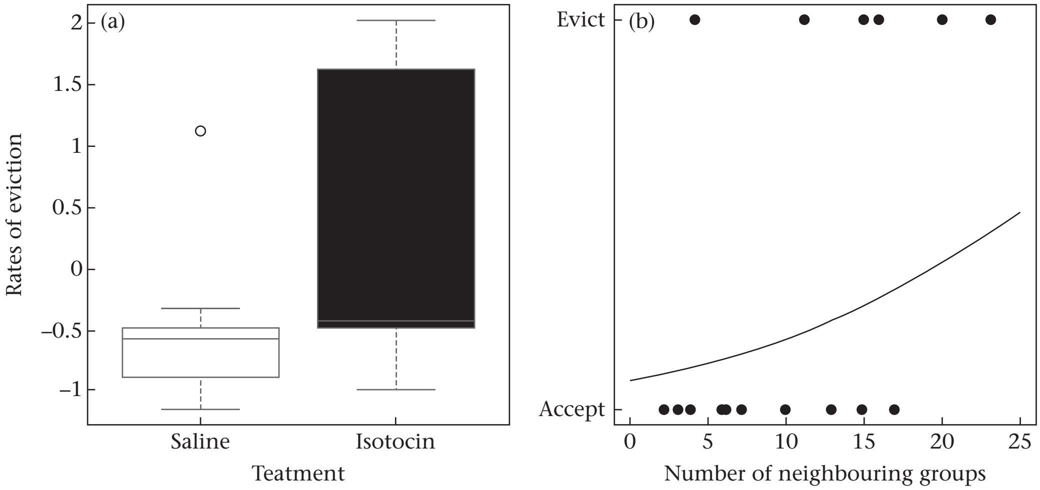

Figure 3. Rates of eviction plotted against (a) hormone treatment (least square mean of the regression model) and (b) the density of surrounding groups. Box plots show the median, first and third quartile, and the 95% confidence intervals (whiskers).

DISCUSSION

We manipulated within-group conflict in the cooperatively breeding cichlid fish *N. pulcher* by removing a high-ranking subordinate and then returning that individual to the group after either a 5 min or a 4 h delay and by injection of IT or a saline control. As predicted, there was no change in aggression received or subordinate submission in control treatments (removals of 5 min). In the removal treatments (removals of ~4 h), group size and number of nearby neighbours were positively correlated with aggression towards the returned subordinate. Returning subordinates also had a higher chance of being evicted from the group when the number of nearby neighbours was high. In the removal treatment, as predicted, we also found that experimentally injecting IT increased the submissiveness of the focal fish, but unexpectedly this manipulation increased the level of aggression that the focal individual received and its likelihood of being evicted. Ours is one of only very few studies that have performed nonapeptide manipulations in a wild animal, and supports the view that the oxytocin family of hormones are important regulators of dominance interactions in fish.

Submissive behaviour is thought to facilitate group stability by streamlining the formation of dominance hierarchies and reducing the need for costly aggressive behaviour by expeditiously settling conflicts between individuals within a social group (Drews, 1993; Wilson, 1975). Thus, well-developed submissive signals may be essential for the emergence and maintenance of stable social groups (Hick et al., 2014; O'Connor et al., 2000; Schenkel, 1967). In this experiment, we predicted that submissive behaviour from the returned subordinate would appease the remaining group members and attenuate conflict within the group (Bergmüller & Taborsky, 2005), reducing the probability of eviction from the group. Surprisingly, we found that despite the increased submissive behaviour shown by the focal fish treated with isotocin in the removal treatment, these fish received more aggression from their group-mates and were less successful in reintegrating themselves into the group. In the previous laboratory study, we saw no effect of IT treatment on the level of aggression from the group towards the focal fish (Reddon et al., 2012). However, Reddon et al. (2012) only briefly removed fish from their group, similar to our removal control treatment. We also did not see any increase in aggression from the group towards fish in our removal control treatment.

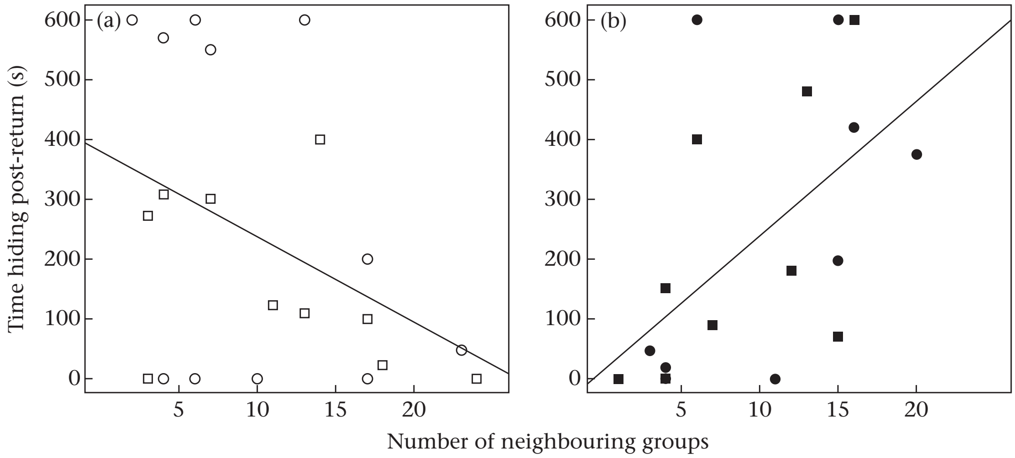

Figure 4. Amount of time that subordinates spent hiding post-return in the (a) saline control treatment and (b) isotocin treatment as a function of the number of neighbouring groups. Squares: 5 min removal (control); circles: 4 h removal treatment. Both slopes differed significantly from the null hypothesis of no effect (GLME: saline: $t_{15} = -4.42$, $P = 0.026$; isotocin: $t_{12} = 2.64$, $P = 0.021$).

We are uncertain why the IT-treated fish received more aggression from their focal group than the fish treated with saline in the removal treatment. It is possible that subordinates that received IT may have responded inappropriately to the social conflict induced by the removal. It is possible that individuals that received IT were inappropriately submissive upon return and therefore, were perceived to be of a lower status than they actually were. Consistent with this idea, other studies that have manipulated physical signals of dominance status (Dey, Dale, & Quinn, 2014; Tibbetts & Dale, 2004) found that reducing an individual's perceived dominance status caused other conspecifics to increase their aggression towards that individual. Therefore, this incongruous submission in our experiment may have increased rank conflict with other similarly sized fish due to the mismatch between body size and the dominance status being signalled behaviourally by the focal fish. However, because we did not track which specific individuals within the social groups were aggressive towards the returned subordinate, we cannot confirm that additional aggression came primarily from similarly sized subordinates. In another experiment on *N. pulcher*, fish that received exogenous IT were more, not less, aggressive to larger and therefore more formidable opponents within staged resource contests (Reddon et al., 2012), supporting the notion that exogenous IT may result in inappropriate social behaviour in some situations.

In addition to the effects of the hormone treatment, we found that within-group conflict also varied with the social landscape, as a higher density of neighbouring groups was correlated with increased aggression towards the returned subordinate and a higher chance of its eviction from the group. Increased aggression and rates of eviction in groups from denser areas may be due to several, nonmutually exclusive patterns. Individuals with bolder, more social, or more aggressive personalities may have a stronger preference for denser social environments compared to shyer and less aggressive individuals, who may prefer less dense social environments (Aplin, Farine, Mann, & Sheldon, 2014; Cote & Clobert, 2007; Cote, Clobert, Brodin, Fogarty, & Sih, 2010). These potential differences in personality due to spatial density could promote increased group aggression towards focal subordinates as well as reduced fidelity of a subordinate to its home group in denser areas (Cote & Clobert, 2007; Dingemanse, Both, van Noordwijk, Rutten, & Drent, 2003; Harrison et al., 2014; Krackow, 2003). It also may foster bolder, more aggressive subordinates that are less willing to hide in response to conflict. This is consistent with our results, although we might also expect subordinate submission to be reduced in denser areas if personality differences are driving spatial variation in behaviour (Sih, Bell, & Johnson, 2004), but we did not observe this effect.

Genetic and behavioural data showing frequent dispersal to neighbouring groups (Bergmüller, Heg, Peer, et al., 2005; Heg et al., 2008; Stiver et al., 2004; Stiver, Fitzpatrick, Desjardins, & Balshine, 2006) strongly suggest that dispersal opportunities increase with neighbour density in *N. pulcher*. Increased movement between groups in denser areas could potentiate within-group aggression by increasing the ability of dominants to attract new subordinates from neighbouring groups. Eviction threats are more credible when subordinates are easily replaced and are reinforced with aggression when subordinates fail to cooperate (Bergmüller, Johnstone, Russell, & Bshary, 2007; Cant & Johnstone, 2009; Kutsukake & Clutton-Brock, 2008a; Noë & Hammerstein, 1994), as was the case with our experimental removals. The importance of these outside options to within-group conflict are particularly acute when within-group relatedness is low (Cant & Johnstone, 2009), which is likely in dense areas with high levels of intergroup movement. Across a variety of taxa, individuals are consistently more aggressive to nonkin than they are to kin (birds: Watson, Moss, Parr, Mountford, & Rothery, 1994; fish: Brown & Brown, 1993; primates: Bernstein & Ehardt, 1986; Butovskaya, 1993) and there is some evidence to suggest that *N. pulcher* can discriminate between individuals on the basis of relatedness (Le Vin, Mable, & Arnold, 2010). Further study is needed to determine whether and how relatedness varies with social density in *N. pulcher*, and what effect relatedness may have on social dynamics within and between groups.

The same biological market dynamics that may have increased eviction and aggression in dense areas may also have increased rates of aggression in larger groups. In a group with few subordinates, each subordinate has a large influence on group productivity and success. Therefore, dominant punishment of these subordinates carries a higher cost and may occur less frequently than in large groups (Kutsukake & Clutton-Brock, 2008a; Noë & Hammerstein, 1994). However, it is also possible that subordinates in larger groups received more aggression simply because there were more group members present (Fischer et al., 2014). In either case, the increased rates of aggression that we found in the larger groups included in our study may not hold for very large groups (larger than those in this study) where dominants may have difficulty recognizing, tracking and punishing particular individual subordinates (Fischer et al., 2014).

We also predicted that subordinates would decrease their submissive behaviour in denser areas of the colony. However, we did not find an effect of group density on submissive behaviour. Alternatively, subordinates may have sought to appease dominant individuals through helping behaviour (territory maintenance, broodcare and territory defence; Taborsky, 1984), as has been previously reported in this species (Balshine-Earn et al., 1998; Bruintjes & Taborsky, 2008; Fischer et al., 2014). However, subordinates in our experiment significantly reduced rather than increased their territory defence against heterospecifics in the post-removal observation compared to the pre-removal observation (Supplementary Table S1). This suggests that subordinates were not using this form of helping behaviour as an appeasement tactic. We could not observe broodcare and territory maintenance behaviours as they occur primarily underneath the territory rocks and so it is possible that subordinates may have been helping while they were perceived to be hiding (anecdotally however, this was not the case). If this were true, then subordinates may have helped more in lower densities (where they hid more), which would be consistent with our hypothesis that subordinates seek to appease dominants more when they have fewer outside options. A laboratory experiment in which broodcare behaviours can be observed directly would be valuable in clarifying this issue.

Individuals may use avoidance behaviour rather than submission to circumvent dominant aggression in wild groups. Submission is an effective appeasement behaviour in *N. pulcher* in a laboratory setting, where individuals are confined to a restricted area with a limited number of places to hide from dominants (e.g. Bergmüller & Taborsky, 2005; Bruintjes & Taborsky, 2008; Hick et al., 2014; Riebli et al., 2011). However, submissive appeasement may not be necessary in the wild if individuals can evade dominant aggression through avoidance within the more complex and less restrictive environment of the field (Matsumura & Hayden, 2006). Our results suggest this, as we found that individuals that hid more tended to have lower rates of submission (controlling for the time spent hiding), but only in the removal trials where we induced a need for dominant appeasement. Similarly, Kutsukake and Clutton-Brock (2008b) found that avoidance, but not submission, reduced the amount of aggression subordinates received from dominant group members in meerkats (*Suricata suricatta*). Subordinates may be less inclined to hide in denser areas because of the greater chances of finding a new group in case of eviction.

Interestingly, we found that the amount of time the focal fish spent hiding after the removal treatment was influenced by an interaction between the number of neighbouring groups and the type of injection that individual received. For focal fish that received saline, the effects of density were as predicted: the amount of time the fish spent hiding was significantly lower in high-density areas. However, we found that when focal fish received IT, the effects were reversed: fish in high densities hid significantly more than fish in low densities. While oxytocin, the mammalian homologue of IT, has generally been known to potentiate prosocial behaviours (Macdonald & Macdonald, 2010), several studies have found that this effect is highly context specific. Oxytocin can actually increase social selectivity and promote social avoidance, particularly towards unfamiliar individuals (Anacker & Beery, 2013). Furthermore, experimental work in *N. pulcher* has shown that exogenous IT reduces the tendency for fish to associate with conspecifics (Reddon et al., 2014), while *N. pulcher* with higher levels of naturally occurring IT in their brains are less likely to show affiliative behaviours (Reddon et al., 2015). Collectively, our results suggest that while IT strongly influences individual behaviour and thus, group response, the influence of IT on within-group conflict and behaviour varies across different social landscapes. Our results demonstrate that the relationship between individual physiology and the social environment is complex, mediated by both the social dynamics within the group as well as the opportunities offered by neighbouring groups.

Acknowledgments

We thank Cyprian Katongo, and the staff of the Tanganyika Science Lodge as well as the Zambian Department of Fisheries for their logistical support of the field research. We thank Kelly Stiver for her advice on mapping *N. pulcher* colonies. We also thank Michael Taborsky and an anonymous referee for valuable feedback on an earlier version of this manuscript. This research was supported by Natural Sciences and Engineering Research Council of Canada (NSERC; RGPIN222854-11), Ontario Innovation Trust and Canadian Foundation for Innovation grants (12805) to S.B. Further funding for the field research was provided by a Journal of Experimental Biology Travelling Fellowship to C.M.O. and McMaster School of Graduate Studies and Canadian Society of Zoologists research grants awarded to A.R.R. J.K.H. was supported by the American Academy of Underwater Sciences. J.K.H. and I.Y.L. were supported by The Ohio State University Fish Systematics Endowment, and the SciFund Challenge. A.R.R. received support from the Margo Wilson and Martin Daly Ontario Graduate Scholarship and is currently supported by the Richard H. Tomlinson Postdoctoral Fellowship, and an NSERC Postdoctoral Fellowship. C.M.O. received support from an E.B. Eastburn Postdoctoral Fellowship from the Hamilton Community Foundation, and is currently supported by an NSERC Postdoctoral Fellowship. S.B. is supported by the Canada Research Chair Program.

Supplementary Material

Supplementary Material associated with this article is available, in the online version, at http://dx.doi.org/10.1016/j.anbehav.2015.03.029.

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