Neighbor Effects: The Influence of Colony-level Social Structure on Within-group Dynamics in a Social Fish

DISSERTATION

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in the Graduate School of The Ohio State University

By

Jennifer K. Hellmann

Graduate Program in Evolution, Ecology and Organismal Biology

The Ohio State University

2016

Dissertation Committee:

Ian M. Hamilton, Advisor
H. Lisle Gibbs
Elizabeth A. Marschall
J. Andrew Roberts
Abstract

In nearly all species, social interactions are highly influential in determining an individual’s success within its environment. In humans, positive social interactions are correlated with increased job success, better health, and reduced stress; in other species, social interactions are critical determinants of disease spread, predation avoidance, and learning. Behavioral ecologists have long been interested in strategic interactions among group members; however, most studies of these do not consider how interactions within a group may change due to the presence of or interactions with other groups. This is problematic, as it is difficult to understand why individuals would cooperate or resolve conflict within their group without understanding the extent to which neighboring groups provide opportunities for individuals to leave their current group. Similarly, it is difficult to understand the extent to which individuals pursue reproductive opportunities within the group without understanding how neighboring groups offer additional opportunities for current and future reproduction. Theoretical models have long suggested that neighboring groups strongly influence individual behavior and reproductive success, but there have been few empirical experiments testing these theoretical predictions.

My dissertation research explores how neighboring groups alter within-group social and reproductive dynamics in *Neolamprologus pulcher*, a cooperatively breeding fish. These fish live in colonies of 2-200 groups, each with a dominant breeding pair and 1-20 subordinates that help maintain the territory and care for offspring. In Chapter 2 and 3, I
demonstrate that the presence of neighboring groups alters within-group social dynamics; specifically, that subordinates demonstrate increased helping behavior when neighbors are present compared to when they are absent and that aggressive, submissive, and affiliative behavioral interactions among group members vary with the presence and familiarity of neighbors. In Chapters 4 and 5, I explore how direct and indirect fitness gains within groups are mediated by colony-level social structure. I find that the degree of reproductive sharing among dominant and subordinate group members (Chapter 4), as well as kinship among group members (Chapter 5), is strongly influenced by the density of neighboring groups, as well as the relative location of a group on the edge versus center of the colony. In Chapters 6 and 7, I demonstrate that neighboring groups can alter dominant tolerance of subordinate group members. In Chapter 6, I found that dominant threats of eviction are triggered more easily in denser areas of the colony, where there is a large pool of potential subordinates that could replace the evicted subordinate. In Chapter 7, I use a game theoretical model to demonstrate that an increased ability of dominants to replace evicted subordinates does not account for the trends observed in Chapter 6. Instead, it is likely that partner choice works in conjunction with negotiation or with variation in helper phenotypes to account for higher eviction thresholds in denser areas of the colony.

Collectively, this research illustrates that the relationship between individual phenotype and the social environment is complex, mediated by social dynamics within the group and opportunities offered by neighboring groups. By examining how multiple levels of the social system interact to influence individual behavior, we can better predict variation in social and reproductive dynamics within and among populations.
Dedication

To my grandfather, Dr. John F. Magill, who first instilled in me a love of nature and science. I will always be grateful for our lessons in the woods and for the example that you set with your constant love and passion for your family, for your work, and for life in general.
Acknowledgements

First, I owe many thanks to my advisor, Ian Hamilton, whose patience, guidance, and advice were invaluable to my development as a scientist. I am tremendously appreciative for his willingness to let me develop and pursue my own research questions. I also thank the members of my committee- Elizabeth Marschall, Andrew Roberts, and Lisle Gibbs- for their help and support throughout my dissertation. I am extremely grateful to a variety of collaborators that have worked with me on this research, including Sigal Balshine, Isaac Ligocki, Constance O’Connor, Adam Reddon, Susan Marsh-Rollo, Lisle Gibbs, Michael Sovic, and Kelly Garvy. Without their guidance and training, I would have not been able to complete this research. I also thank the members of the Hamilton lab for their feedback and assistance over the years, Jose Diaz for help with the genetic analyses, and Cody Dey for aid with network analysis. Finally, I am grateful to my wife, Christa, and my family for their constant support and encouragement. This dissertation research was supported by an OSU University Fellowship, OSU Presidential Fellowship, Alumni Grants for Graduate Research and Scholarship (AGGRS), OSU Fish Systematics Endowment, SciFund Challenge, Animal Behavior Society, and American Academy of Underwater Sciences.
Vita

2006........................................... Perkiomen Valley High School, Collegeville, PA

2010........................................... B.S. Biology, B.A. Spanish, Messiah College

2011-2012 ......................... University Fellow, The Ohio State University

2012-2015 ......................... Graduate Teaching Associate, The Ohio State University

2015-2016 ......................... Presidential Fellow, The Ohio State University

Publications

Hellmann JK, Sovic MG, Gibbs HL, Reddon AR, O’Connor CM, Ligocki IY, Marsh-Rollo SE, Balshine S, Hamilton IM. Accepted. Within-group relatedness is correlated with colony-level social structure and parentage in a social fish. Molecular Ecology


**Fields of Study**

Major Field: Evolution, Ecology and Organismal Biology
Table of Contents

Abstract .................................................................ii
Dedication .................................................................iv
Acknowledgments .......................................................v
Vita ...........................................................................vi
Publications......................................................................vi
Fields of Study................................................................vii
Table of Contents..............................................................viii
List of Tables ...................................................................xi
List of Figures...................................................................xiv
Chapter 1: Introduction......................................................1
Chapter 2: The Presence of Neighbors Influences Defense Against Predators in a Cooperatively Breeding Cichlid............................7
  Introduction...................................................................8
  Methods.......................................................................11
  Results........................................................................14
  Discussion....................................................................20
Chapter 3: The Presence and Familiarity of Neighbors Influences Intragroup Network Dynamics and Sexual Conflict in a Group-living Fish...........................................25
  Introduction...................................................................27
List of Tables

Table 1: Results of generalized linear mixed models (negative binomial distribution) testing the effect of treatment (isolated or neighbors), the number of subordinates in the group, aggression received and affiliative behavior received on behavioral interactions between the dominant male and female pair.  ................................................................. 41

Table 2: Results of ERGM fit for behavioral networks for isolated groups (Days 30-39), groups with new neighbors (Days 40-49), and groups with established neighbors (Days 70-79). ..........................................................42

Table 3: Results of ERGM fit for difference networks comparing isolated groups (Days 30-39), groups with novel neighbors (Days 40-49), and groups with established neighbors (Days 70-79). Negative estimates indicate that behaviors occurred more frequently in the earlier treatment (listed first), while positive estimates indicate that behaviors occurred more frequently in later periods (listed second). .................................................................43

Table 4: Allocation of paternity within groups. Test statistics (with df) and p-values from full models measuring the effect estimates of location (center versus edge), density of neighboring groups, group size, relative size of dominant male to dominant female ([dominant male SL-dominant female SL]/dominant male SL), and the number of male subordinates on the number of offspring assigned to dominant and subordinate males within the sampled group (GLMER) and number of fathers contributing to the brood (CLM). Total number of offspring within each brood was included as a fixed effect to control for variation in brood size. Asterisks indicate significance, assessed at α=0.05...67

Table 5: Allocation of maternity within groups. Test statistics (with df) and p-values from full models measuring the effect estimates of location (center versus edge), density of neighboring groups, group size, relative size of dominant male to dominant female ([dominant male SL-dominant female SL]/dominant male SL), and the number of female subordinates on the number of offspring assigned to dominant and subordinate females within the sampled group (GLMER) and the total number of mothers contributing to the brood (CLM). Total number of offspring within each brood was included as a fixed effect to control for variation in brood size. Asterisk indicate significance, assessed at α=0.05...........................................................68

Table 6: Genetic characteristics of the six loci used to run the relatedness analysis based on microsatellites. Shown are observed (H_{obs}) and expected (H_{exp}) heterozygosity, the polymorphic information contents (PIC), and the estimated frequency of null alleles for
each locus. Heterozygosity, PIC, and null frequencies were calculated using CERVUS 3.0 based on genetic data from the 54 unrelated dominants from the reduced dataset.…93

Table 7: Best-fit models illustrating effects on aggression received from the group (GLMM), submission given by the focal subordinate (GLMM), time spent hiding post-return (linear mixed effects model) and rates of subordinate eviction/re-acceptance into the group (logistic regression). Dashes refer to factors that were not included in the best-fit models. Significant $P$ values ($\alpha<0.05$) are shown in bold. ............................ 117

Table 8. Genetic characteristics of the five loci used to assign parentage. Shown are observed ($H_{\text{obs}}$) and expected ($H_{\text{exp}}$) heterozygosities, the polymorphic information contents (PIC), non-exclusion probabilities (ability to identify the real parent or parental pair out of the population given allele frequency and diversity), and the estimated frequency of null alleles for each locus. Values were calculated using CERVUS 3.0 with a sample of 74 unrelated individuals (all parents) from the population. ............................ 179

Table 9. Means and standard errors of the measured within-group characteristics (territory size [m$^2$], group size, and the number of reproductively mature male and female subordinates per group) and measured individual characteristics of the dominants within the group (dominant male and female standard length [mm] and the relative difference in size between the dominants ([dominant male SL-dominant female SL]/dominant male SL). None of these characteristics differed significantly between the edge and the center of the colony or with the density of neighboring groups. ............................ 179

Table 10. Test statistics (with df) and $p$-values from full models measuring the effect estimates of location (centre versus edge), density of neighbouring groups, group size, relative size of dominant male to dominant female ([dominant male SL-dominant female SL]/dominant male SL), and the number of male subordinates on the number of offspring assigned to dominant and subordinate males within the sampled group (GLMER) and number of fathers contributing to the brood (CLM). These are data from Table 2 excluding two groups in which the dominant male gained no paternity. All significant results were robust to these exclusions. ............................ 180

Table 11. Test statistics (with df) and $p$-values from full models measuring the effect estimates of location (centre versus edge), density of neighbouring groups, group size, relative size of dominant male to dominant female ([dominant male SL-dominant female SL]/dominant male SL), and the number of female subordinates on the number of offspring assigned to dominant and subordinate females within the sampled group (GLMER) and the total number of mothers contributing to the brood (CLM). These are data from Table 1 excluding the two groups in which the dominant female gained no maternity. All significant results were robust to these exclusions, except for the observed effect of the relative size difference between the dominant male and female on the number of offspring assigned to dominant female. ............................ 180
Table 12. Comparison between pre- and post-removal behaviour (Mann–Whitney U tests) for all experimental treatment combinations.. 182
List of Figures

**Figure 1:** Least squared means for group overt and restrained aggression against the predator between neighbor absent and neighbor present treatments. Means and 95% confidence intervals were calculated from square-root transformed data and backtransformed................................................................. 17

**Figure 2:** Least squared means for total aggression against the predator between neighbor absent and neighbor present treatments for dominant females, dominant males, and subordinates. Means and 95% confidence intervals were calculated from square-root transformed data and backtransformed................................................................. 18

**Figure 3:** Least squared means for total aggression against the predator for familiar versus unfamiliar neighbors for dominant females, dominant males, and subordinates. Means and 95% confidence intervals were calculated from square-root transformed data and backtransformed................................................................. 19

**Figure 4:** Diagram of experimental set-up dictating observation periods (Days 30-39, Days 40-49, and Days 70-79) .................................................................................................................... 34

**Figure 5:** Dominant male and female aggression toward their mate, toward subordinates within their group, and toward the neighboring group for isolated groups (Days 30-39), groups with new neighbors (Days 40-49), and groups with established neighbors (Days 70-79) ........................................................................................................ 44

**Figure 6:** Dominant male affiliation toward the dominant female (white), dominant female affiliation toward the dominant male (light gray), and dominant female submission toward the dominant male (dark grey) for isolated groups (Days 30-39), groups with new neighbors (Days 40-49), and groups with established neighbors (Days 70-79). Data presented are total counts of behaviors across the 10 observations in each treatment period, with 95% confidence intervals......................................................... 45

**Figure 7:** Aggressive supernetwork structure of for isolated *N. pulcher* groups (Days 30-39), groups with new neighbors (Days 40-49), and groups with established neighbors (Days 70-79). Node orientation was determined the Fruchterman-Reingold algorithm. Larger nodes represent dominant individuals, red nodes represent females, and blue nodes represent males. Thicker edges indicate that more aggression was exchanged between a given dyad. ........................................................................................................ 46
Figure 8: The percent of offspring (pooled across all 36 sampled groups) belonging to the dominant male or female, (black) and to subordinate males and females in the social group (light grey), as well as all other instances of extra-group reproduction (in dark gray, a combination of reproduction assigned to known individuals outside the sampled group and reproduction that was unable to be assigned)........................................................................................................ 69

Figure 9. A) In the 12 groups on the edge of the colony, the number of offspring mothered by the dominant female decreased as the number of female subordinates in the group increased (GLMER: Z_{6}=-2.97, p=0.003). B) In contrast, in the 24 groups in the center of the colony, the number of offspring mothered by the dominant female increased as the number of female subordinates in the group increased (GLMER: Z_{16}=3.38, p<0.001) ........................................................................................................ 70

Figure 10. Across all 36 sampled groups, dominant females mothered more offspring (after controlling for variation in brood size) when they were mated with a large male, both in terms of absolute size (standard length [SL], as shown in this figure) and in terms of relative size in relation to the dominant female’s size (Table 5). Data presented are least squared means of the regression model testing predictors of the number of offspring mothered by the dominant female. ........................................................................................................ 71

Figure 11: Partial map of the 7 sampled colonies (and one additional unsampled colony), with unsampled groups as grey dots and sampled groups as black dots. After removing 3 individuals from our dataset due to low sequencing coverage, we analyzed a total of 22 dominants and 37 subordinates across 11 edge groups, and 37 dominants and 72 subordinates across 20 center groups. Lines connect kin found in different groups within the same colony, as well as different groups in different colonies. Solid lines indicate full-siblings/offspring between two groups and dashed lines indicate half-siblings between two groups. Depths of the colonies are identified on the bars lining the colony map Numbers next to the colonies indicate groupings of the colonies into 4 smaller populations for analysis. Note that distances between the colonies on the figure are not to scale and there are additional unsampled groups that are not depicted on the map........94

Figure 12: Mean relatedness (± standard error) between dominants and subordinates for groups in the center of the colony versus on the edge of the colony. Subordinates were significantly more related to the dominant male in their group when groups were located in the center of the colony compared to the edge of the colony, but relatedness between dominant females and subordinates did not vary significantly between groups on the center and edge of the colony.................................................................95

Figure 13: The number of neighboring groups within a 3m radius, plotted against the relatedness values of subordinates to the dominant female within their group. As the density of neighboring groups increased, subordinates were significantly less related to dominant females within their group.................................................................96
Figure 14: Using RADseq, we identified unrelated individuals from different groups (white), half-sibs from different groups (light grey), and full-sibs/offspring from different groups (dark grey). Data presented are means with 95% confidence intervals. RADseq techniques significantly improve the precision of relatedness estimates compared to microsatellites, which provided much larger confidence intervals.

Figure 15. 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).

Figure 16. 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 17. 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).

Figure 18. 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 neighboring groups. Squares: control removal; circles: treatment removal. Both slopes differed significantly from the null hypothesis of no effect (GLME: saline: $t_{15} = -4.42, P = 0.026$; isotocin: $t_{12} = 2.64, P = 0.021$).

Figure 19: The function $(p_e = 1/2 + 1/2 \ast \tanh ((p - h) \ast k))$ specifying the probability of eviction $(p)$ given the level of helping behavior $(h)$.

Figure 20: The level of subordinate helping (solid line) and threshold of eviction (dashed line) as they vary with: A) the probability of dominants replacing evicted subordinates $(n)$ when the cost of subordinates to dominants $(s)$ is 20%, 40% and 60% of group productivity $(G)$, B) the cost of helping $(c)$, C) the probability of a subordinate becoming dominant if in a group (black) or if evicted (grey), and D) subordinate mortality within the group $(\mu_g)$. For all panels, parameters that are not varied are $G=1, c=0.1, m=0.1, s=0.4, k=50, e_d=0.05, e_s=0.05, \mu_g=0.2, \mu_e=0.4, n=0.1, a_g=0.5, a_e=0.25$.

Figure 21: Values of helping behavior and eviction threshold across all values of $a_g$ (A) and $a_e$ (B) when group productivity is low and when the probability of replacing the subordinate $(n)$ is 20%, 40% and 60%. When helping behavior is much lower than the eviction threshold, the group is likely to disband. For all panels, parameters that are not varied are $G=0.5, c=0.1, m=0.1, s=0.4, k=50, e_d=0.05, e_s=0.05, \mu_g=0.2, \mu_e=0.4$, and $n=0.1$. $a_g$ and $a_e$ vary independently of each other.

Figure 22: As $k$, or the steepness of the probability of eviction curve, increases, the threshold of eviction (dashed line) approaches the level of subordinate help provided...
(solid line). The probability of eviction (red dotted line) remains near zero across all values of k. ..........................................................183

**Figure 23:** The level of subordinate helping (solid line) and the threshold of eviction (dashed line), as it varies with the immediate cost of eviction to subordinates (pink) and dominants (blue) .........................................................................................................................184
Chapter 1: Introduction

For nearly all organisms, social interactions are key determinants of lifetime success, influencing the quantity and quality of breeding opportunities (McDonald 2007, Ryder et al. 2009), adult longevity (Barocas et al. 2011), offspring survival (Silk et al. 2009), body condition (Pinter-Wollman et al. 2009), and group stability (Flack et al. 2006). While we expect individuals to adopt patterns of interactions that optimize their fitness, the fitness consequences of social interactions can vary based on the social environment in which they occur (Sih et al. 2009). Consequently, individual decision-making cannot be understood without accounting for variation in the social environment or in individual characteristics (i.e. sex, status) that may influence the decisions available to an individual or the fitness consequences of particular behaviors.

In group-living species, group-level characteristics have a profound influence on the social and reproductive dynamics among group members. For example, social and reproductive dynamics within a group are altered by the size of the group (Madden et al. 2009), the sex of group members (Kutsukake and Clutton-Brock 2008), relatedness among group members (Nam et al. 2010, Chiyo et al. 2011), and the relative size of group members (Hamilton et al. 2005). However, despite the importance of group-level characteristics in dictating within-group dynamics, social groups usually do not occur in isolation. Instead, social groups are often embedded in a larger social structure where the
presence of other groups can also influence how individuals behave within their own
group. Specifically, neighboring groups can threaten the territory or resources of an
established group and may incentivize group members to quickly resolve or reduce
conflict within their own group in order to facilitate cooperation in between-group
conflict (Radford 2008, Bruintentjes et al. 2016). Further, neighboring groups can offer
opportunities for individuals to move between groups (Heg et al. 2008a), which can
reduce the stability of group composition and dilute kinship among current group
members. Finally, neighboring groups offer opportunities for extra-pair fertilizations
(Petrie and Kempenaers 1998, Westneat and Stewart 2003), which may alter the ways in
which individuals pursue reproductive opportunities within their own group, as well as
the extent to which dominants police subordinate reproduction within their own group.

Theoretical models suggest that neighboring groups should alter within-group
reproductive and social dynamics (Reeve 1998, Johnstone and Cant 1999, Cant and
Johnstone 2000, Reeve 2000, Hamilton 2004, Buston and Zink 2009), but predictions
made by theoretical models have far outnumbered empirical studies that explore how the
broader social context influences within-group dynamics in group-living species. In this
dissertation, I explore how the presence, density, and location of neighboring groups
influence social interactions among group members (Chapters 2-3), the degree of
reproductive sharing and kinship among dominant and subordinate group members
(Chapters 4-5), and the stability of social groups (Chapters 6-7). To do this, I use the
cooperatively breeding fish *Neolamprologus pulcher*, which is native to Lake Tanganyika,
East Africa. Groups defend rocky territories clustered in colonies of 2-200 groups; each
group is composed of a dominant breeding pair and 1-20 subordinate helpers that care for the offspring of the dominant breeders (Wong and Balshine 2011a).

In Chapter 2, I conducted a laboratory experiment in which I examined group defense against a heterospecific predator when *N. pulcher* groups were isolated versus when groups could see their neighbors. I found that subordinates, but not dominants, demonstrated higher levels of defense when neighbors were present compared to when they were absent, particularly when those neighbors were unfamiliar. As defense against predators is costly and important to group survival (Grantner and Taborsky 1998), subordinates may increase their defense in the presence of neighbors as a means of signaling their ability to contribute to that group. This is expected to be more important for subordinates than dominants, as subordinates often disperse to neighboring groups to breed (Stiver et al. 2007, Stiver et al. 2008) while dominants depend on the productivity of the group for most of their current and future reproductive fitness (Brouwer 2005, Awata et al. 2010).

In Chapter 3, I further examined the effects of neighbors on group dynamics by analyzing how social interactions change with neighbor presence in laboratory groups of *N. pulcher*. Aggression and submission between mates was higher when groups were isolated, but aggression between dominants and subordinates was relatively more frequent when groups had neighbors. This suggests that there is greater conflict between the dominant pair in the absence of neighbors, but greater conflict between dominants and subordinates when neighbors are present. Additionally, I found that the dominant males maintained a prominent role in aggression networks across all time periods, but
that dominant males play a less prominent role in affiliative intragroup networks when neighbors are present.

In Chapter 4 and 5, I found that both group and colony-level social structure influenced patterns of reproductive sharing and kinship among group members in wild *N. pulcher* groups. The frequency of subordinate male reproduction varied with both within-group and between-group attributes: a subordinate male was more likely to reproduce within his group when his group contained many subordinate males and when his group was located in a denser area of the colony. Further, I found that the influence of some group-level characteristics was dependent upon colony-level social structure. Dominant males and females lost more parentage in larger groups and groups with more reproductively mature subordinates, but only when their group was located on the edge of the colony (Chapter 4). In Chapter 5, I demonstrate that within-group reproductive patterns are correlated to patterns of kinship among group members, as subordinates who were closely related to their same-sex dominant were more likely to reproduce within their group. Further, I found that colony-level social structure influenced within-group relatedness. Subordinates at the colony edge were less related to dominant males in their group than were subordinates in the colony center, suggesting a shorter tenure in the breeding position for males at the edge of the colony. These results, in conjunction with those in Chapter 4, suggest that dominant males, but not dominant females, face a cost of living on the edge of the colony. Further, subordinates may have altered incentives to reproduce and help in different areas of the colony due to variation in kin-selected benefits of helping.
In Chapter 6, I experimentally induced group instability by removing subordinates from wild group of *N. pulcher* for 4-6 hours, which simulates a dereliction of helping behavior (Balshine-Earn et al. 1998). I found that groups in denser areas of the colony (i.e. groups with a greater number of neighboring groups) were more aggressive toward the subordinate when it was returned to the group and were more likely to evict the returned subordinate. Having many surrounding groups is expected to increase the ability of dominants to replace subordinates with a disperser from a nearby group (Heg et al. 2008a). Therefore, in periods of long removals where helpers are not contributing to group productivity, the reduced cost to losing current subordinates could increase dominant aggression in denser areas (Noë and Hammerstein 1994).

In Chapter 7, I extend the results of Chapter 6 by developing a game theoretical model examining how variation in the relative costs of eviction for dominants influences the degree of helping behavior exhibited by subordinates. I found that dominant ability to replace evicted subordinates (i.e. partner choice) alone is not the primary mechanism regulating market dynamics in group-living species, as increased opportunities for dominants to replace evicted subordinates resulted in decreased subordinate helping behavior and reduced eviction thresholds. Therefore, it is likely that partner choice, in tandem with other mechanisms such as negotiation, plasticity, or phenotypic variation in subordinate cooperative tendencies, regulates dynamics observed in empirical systems.

Collectively, throughout this dissertation, I use a mix of laboratory and field experiments, as well as a game theoretical model, to demonstrate that understanding dynamics in group-living and/or cooperatively breeding species is difficult without
accounting for the social structure beyond the level of the group. The extent to which individuals cooperate and mitigate conflict within their group is strongly influenced by their ability to leave or reproduce outside of the group, and thus, studies exploring the evolution of cooperation must consider how individuals make decisions on the basis of fitness that can be gained from their current group versus from neighboring groups.
Chapter 2: The Presence of Neighbors Influences Defense Against Predators in a Cooperatively Breeding Cichlid

Abstract: Although behavior is often examined in a dyadic or group context, behavioral interactions are also influenced by the broader social context. Interactions with neighboring groups may provide information about the local environment or may offer important reproductive opportunities. Considering the potential fitness consequences of intergroup interactions, particularly for territorial species that have long-term relationships with neighboring groups, we expect individuals will alter their behavior based on access to surrounding groups. We used a cooperatively breeding cichlid Neolamprologus pulcher to determine if individuals vary their response to a predator when neighbors were present versus absent. We found no difference in dominant behavior between neighbor-present and neighbor-absent treatments, but subordinates increased their defense against the predator when neighbors were present, particularly when the neighbor was unfamiliar. Subordinates may change their behavior when neighbors are present in order to signal to or cooperate with neighboring individuals, but may also be responding to the change in intragroup dynamics that can result from the presence of neighboring conspecifics. This study illustrates the importance of considering the effect of neighboring groups on individual behavior, given that intergroup dynamics can influence behaviors that do not directly involve conspecific interactions.
Introduction

For nearly all organisms, conspecific interactions are a strong determinant of how successful an individual is within its environment. These interactions have traditionally been studied in a dyadic or group context, where it has been shown that the composition and size of the group strongly influences individual behavior (Kutsukake 2006, Kutsukake and Clutton-Brock 2008, Hamilton and Ligocki 2012), survival (Heg et al. 2004a, Barocas et al. 2011), and reproduction (Heg 2008, Heg and Hamilton 2008).

While most individuals spend the majority of their time interacting with members of their immediate social group, individuals in many social species also interact with neighboring groups. Intergroup interactions provide valuable opportunities to gain information that may not be available from interacting with individuals within their group, including information about local conditions such as predator threats (Heg et al. 2008a), breeding opportunities (Lazaro-Perea 2001), resource availability (Krause et al. 2007), and conspecific competition (Sicotte and Andrew 2004, Sih et al. 2009). Consequently, individuals often seek out interactions with neighboring groups (Doolan and Macdonald 1996, Bergmüller et al. 2005a, Young et al. 2005, Kesler and Haig 2007). However, neighboring groups can also have an indirect influence on individual behavior by altering intragroup dynamics (Lazaro-Perea 2001, Radford 2008) and the opportunities available to individuals (e.g. dispersal, mating; Westneat and Sherman 1997, Bergmüller et al. 2005a, Heg et al. 2008a). This suggests that the presence of neighboring groups may change how individuals make decisions in a given social environment, particularly for behaviors that have shared fitness implications for individuals in neighboring groups.
Antipredator behavior is important for both intragroup and intergroup dynamics on an ecological and social scale. Within a group, antipredator behavior is highly important in reducing mortality (Caro 2005) but it can also serve a social role as a cooperative behavior used to appease more dominant individuals (Heg and Taborsky 2010). In terms of intergroup dynamics, individuals often monitor neighboring groups for antipredator behavior to know when to flee from predators (Heg et al. 2008a) and the presence of neighbors has been shown to reduce antipredator effort in mating pairs by allowing individuals to exploit the defense effort of neighbors (Kazama and Watanuki 2010, Schadelin et al. 2012). While this demonstrates that individuals adjust their behavior when neighbors are present, the social system is expected to influence how individuals adjust their behavior in the presence of neighbors.

We used the group-living cichlid *Neolamprologus pulcher* to evaluate if and how individuals within a group shift the quantity and intensity of their aggressive interactions with the predator when neighbors were present versus absent. While several recent studies (Kazama and Watanuki 2010, Schadelin et al. 2012) have found that neighbors reduce individual defense against predators in colonies of pair-living animals, there are two important ways in which social dynamics in a system with mating pairs are expected to be very different than the dynamics in a social system with large groups defending permanent territories. First, mated pairs differ in within-group social dynamics, as there are fewer potential partners for group cooperation and dominance relationships. Second, opportunities for movement are more common and more important in larger groups with
non-breeding individuals than they are for mating pairs (Koenig et al. 1992, Kesler and Haig 2007).

We predicted that *N. pulcher* individuals would increase their defense effort in the presence of neighbors and that subordinates should be particularly sensitive to the presence of neighbors. These predictions arose for two main reasons. First, because group members, particularly subordinates, have opportunities to move to nearby groups, they are expected to benefit from using antipredator behavior to convey information, such as fighting or helping ability, willingness to cooperate, suitability as a mate, and individual identity, to nearby groups because it should have future implications for their relationship with these groups (Sicotte and Andrew 2004, Kesler and Haig 2007, Young et al. 2007). Second, the presence of neighboring groups is expected to change social dynamics within the focal group by reducing dominant tolerance of subordinate behavior (Hellmann et al. 2015b), forcing subordinates to invest more energy into predator defense to avoid punishment from more dominant individuals (Bergmüller and Taborsky 2005). We also predicted that defense against predators should be higher for unfamiliar neighbors than for familiar neighbors, given that social vigilance between groups is highest when neighbors are unfamiliar (Stamps 1991) and that increased antipredator behavior may improve the rate at which unfamiliar neighbors learn to recognize specific individuals (Dale et al. 2001). This is critical due to the important benefits to having familiar neighbors (dispersal, safe havens; Bergmüller et al. 2005a). The presence of neighboring groups is expected to influence the range of options available to a given individual. Quantifying if and how neighboring groups influence individual behavior will elucidate
the extent to which the larger social context should be considered when examining intragroup dynamics.

**Materials and methods**

*N. pulcher* is a cooperatively breeding cichlid native to Lake Tanganyika, East Africa. Groups defend rocky territories clustered in colonies of 2-200 groups (Stiver et al. 2007); each group is composed of a dominant breeding pair and 1-20 subordinate helpers that care for the offspring of the dominant breeders (Taborsky and Limberger 1981, Balshine et al. 2001). Subordinates form size-based dominance hierarchies and queue for the opportunity to gain breeding status in their current territory (Wong and Balshine 2011a). However, many individuals cannot gain breeding status in their natal territories (Stiver et al. 2006) and consequently disperse to neighboring territories to fill vacant breeding positions or assume a more dominant helping position in another group (Stiver et al. 2004, Bergmüller et al. 2005a, Heg et al. 2008a).

13 groups of *N. pulcher* were housed in 114-L aquaria with one half of an inverted flowerpot as a shelter and potential breeding site. To mirror natural conditions, a 12:12h light: dark schedule was maintained for the duration of the experiment and water temperatures were kept constant at 27°C ±1°C. Fish were fed daily and ad libitum with either TetraMin flakes (5 days a week) or frozen *Daphnia* or *Artemia* (twice per week). Groups were established more than a year prior to this experiment. Dominant breeders were wild caught or F1 offspring of wild-caught fish from Kipili, Tanzania; all subordinates were descendent offspring of the dominant breeding pair. Group size varied
from 3-10 individuals. All groups had at least one subordinate >25mm standard length 
(SL, the length from the tip of the snout to the base of the caudal fin) and most groups 
also had juvenile individuals (15-24.5 SL) which were included in determining group size, 
but were not observed during the behavioral observations. Prior to behavioral 
observations, all individuals >25mm SL were measured and reproductive mature 
individuals were sexed.

In a randomized order, we exposed focal groups to a predator when neighbors 
were present and when neighbors were absent. In neighbor present trials, focal groups 
were able to see and interact with the group in the neighboring aquarium; in neighbor 
absent trials, barriers were placed between the two aquaria so that groups could not see 
the neighboring group. Prior to conducting behavioral observations and between 
treatments, groups were given seven days to adjust to the absence or presence of 
neighbors. In addition to examining the effect of the presence of neighbors, we also 
evaluated the effect of the familiarity of neighbors. For trials in which focal groups could 
interact with neighboring groups, half of the groups were exposed to groups that they had 
been neighbors with for more than a year prior to this experiment, whereas the other half 
of groups were exposed to novel neighboring groups. All neighbors were unrelated to the 
focal group.

We presented a heterospecific fry predator *Altolamprologus compressiceps* to the 
focal group to induce antipredator behavior. Social monitoring occurs in other species 
when predation pressure is moderate, but not when it is high (Hirsch 2002); therefore, by 
selecting fry predators, predators represent a moderate threat that still elicits predation
defense, but at a lower rate that still permits social monitoring and intergroup interactions. Predators were measured and individually marked prior to the experiment (we used 6 predators ranging from 34-43mm SL). A randomly assigned predator was introduced into the aquarium in a clear glass jar. Predators were captured and given a two-minute acclimation period before the jar was placed on the opposite side of the aquarium as the neighboring group; when the group was isolated, the jar was placed in the same position for standardization. We also conducted a control treatment in which we presented an empty jar to the focal group to ensure that individuals were responding to the predator rather than the jar itself. During control observations, the jar was filled with distilled water and the same experimental procedure was followed. Groups were given one minute to adjust to the presence of the jar before behavioral observations began.

Fifteen minute behavioral observations were conducted between the hours of 1200 and 1500. Observation periods were video recorded and were later scored to ensure a complete and non-biased documentation of all behaviors. We recorded all aggressive interactions of dominant breeders and subordinate helpers >25mm with the predator, as well as all aggressive, affiliative, and submissive interactions within the group and with neighbors. Behavior for juveniles (individuals <25mm) was not recorded, as fish under that size rarely displayed to the predator or neighboring group and many behaviors of small fish could not be reliably identified on recordings. Aggressive interactions were divided into overt aggression (any aggressive behavior with physical contact: ram, bite, tail beat, mouth fights) and restrained aggression (any aggressive display without physical contact: chase, fin raise, puffed throat, head shake, hook and ‘J’ display, and
aggressive postures; see Sopinka et al. (2009) for a complete ethogram of *N. pulcher* behavior). All methods were approved by The Ohio State University chapter of IACUC (protocol ID 2008A0095).

**Statistical Analysis.** We analyzed the predictors of predator defense levels using linear mixed-effects models (R 2.14.2, package nlme). All aggressive behaviors were pooled into two categories: overt or restrained. For most of the analyses, total aggression towards the predator (sum of overt and restrained aggression) was used as the dependent variable because overt aggression was fairly uncommon. Counts of aggression were square root transformed for normality. Random effects of predator identity, group, and individual identity (nested within group) and fixed effects of sex/status (dominant males, dominant females, or subordinates), treatment, and type of neighbor (familiar or unfamiliar) were tested in the models. The number of subordinates and group size (number of juveniles and subordinates) were tested as additional fixed effects and did not influence the relationship between treatment and sex/status. Predator size was also considered as a random effect instead of predator identity, but including predator size significantly worsened the fit of the model in comparison to predator identity. Interactions were tested and non-significant interactions were removed from the models.

**Results**

No aggressive response was produced in control trials with an empty jar; however, aggressive responses were elicited from the group when predators were present (Welch’s
t-test; t=−5.48, df=80.63, p<0.001). There was no effect of treatment on total group aggression towards the predator (sum of overt and restrained aggression; F1,19=0.74, p=0.40); however, when considering overt and restrained aggression separately, we found significantly higher levels of restrained aggression toward the predator when neighbors were present (F1,19=5.65, p=0.015; Fig. 1). Group size was also a significant predictor of total group defense against the predator, with increased defense in larger groups (F1,18=8.34, p=0.01). However, the number of subordinates (the individuals for whom predation defense was recorded) was not as strongly correlated with group defense against predators (F1,19=3.74, p=0.07).

In treatments with no neighbors, there was no effect of sex/status on total aggression toward the predator (F2,31=0.57, p=0.57). When exposed to neighbors, dominant males and females still showed equivalent levels of aggression to each other, as treatment had no impact on either dominant male (F1,18=0.02, p=0.88) or female (F1,18=0.40, p=0.54) defense (Fig. 2). Subordinates were significantly more aggressive toward the predator than dominants when neighbors were present (F2,29=11.14, p<0.001), due to a significant increase in defense effort compared to non-neighbor treatments (F2,29=4.70, p=0.04). In treatments with neighbors present, there was no impact of neighbor familiarity on dominant males (F1,5=0.63, p=0.46) or females (F1,5=0.01, p=0.91), but unfamiliar neighbors elicited significantly higher levels of aggression towards the predator for subordinates (F1,5=7.21, p=0.04; Fig. 3). We found no difference in interactions between the focal group and the neighboring group when neighbors were familiar versus when they were unfamiliar (F1,31=1.44, p=0.16). The number of
interactions (aggressive and affiliative) with the neighbor was not correlated with aggression toward the predator for dominant males ($F_{1,5} = 0.45, p = 0.53$), dominant females ($F_{1,5} = 2.11, p = 0.21$), or subordinates ($F_{1,5} = 0.57, p = 0.48$).

When examining intragroup dynamics, we found no difference in dominant aggression towards subordinates when neighbors were present versus absent ($F_{1,18} = 0, p = 1.00$), nor in dominant male aggression towards the dominant female ($F_{1,18} = 0.96, p = 0.36$). There was a slight, but non-significant decrease in subordinate submission (per aggressive act received) to dominants when neighbors were present ($F_{1,18} = -1.71, p = 0.11$).
Figure 1: Least squared means for group overt and restrained aggression against the predator between neighbor absent and neighbor present treatments. Means and 95% confidence intervals were calculated from square-root transformed data and backtransformed.
Figure 2: Least squared means for total aggression against the predator between neighbor absent and neighbor present treatments for dominant females, dominant males, and subordinates. Means and 95% confidence intervals were calculated from square-root transformed data and backtransformed.
Figure 3: Least squared means for total aggression against the predator for familiar versus unfamiliar neighbors for dominant females, dominant males, and subordinates. Means and 95% confidence intervals were calculated from square-root transformed data and backtransformed.
Discussion

We found that the presence of neighbors had no effect on dominant male and female behavior, but did increase subordinate aggression toward the predator (Fig. 2), particularly when the neighbor was unfamiliar. There seems to be no evidence of a dilution effect, as there was no reduction in defense against the predator when neighbors were present (Fig. 1). It is possible that a dilution effect could have the opposite effect. The presence of additional individuals may reduce the perceived threat of predators and increase subordinate defense by increasing the safety of defending against the predator; however, this seems unlikely given that evidence of ‘dilution effects’ in previous experiments have resulted in the reduction of defense effort (Kazama and Watanuki 2010, Schadelin et al. 2012) and individuals showed an increase in restrained, but not overt aggression, which is the safer form of aggression because it can be done from a distance.

There are several non-mutually exclusive hypotheses that may explain why subordinates, but not dominants, increase their defense against the predator when neighbors are present. Subordinates could be reacting directly to the presence of neighbors by helping in order to gain a future benefit from a neighboring group. This benefit could come in several forms. First, by increasing their defense against the predator, subordinates could increase their chance of being accepted as a future group member or mate by signaling their willingness and/or ability to contribute to group survival (Dugatkin and Godin 1992, Dale et al. 2001, Wong and Balshine 2011a). Defending against predators would be an ideal signal of the value of a particular individual to a group because this behavior is both costly (Grantner and Taborsky 1998)
and important to group survival. Because it is costly, however, the amount of effort an individual puts into this signaling should correlate with the amount of potential reproductive fitness that can be gained from nearby groups. As most subordinate individuals disperse to neighboring groups to breed (Stiver et al. 2007), whereas dominant individuals depend on the productivity of the group for most of their current and future reproductive fitness (fish: Brouwer 2005, Awata et al. 2010; insects: Gadagkar 1990, Shreeves and Field 2002; mammals: Smith et al. 2006; birds: (Kingma et al. 2010), we expect intergroup signaling to be more important for subordinates than it is for dominants.

Second, subordinates may also benefit from defending against the predator when neighbors are present because it may incentivize others to defend in the future (reciprocal altruism; Trivers 1971). Reciprocal altruism would be likely to occur in colonial species, where groups are expected to have long-term relationships (Trivers 1971, Roberts 2005). Cooperation is expected to be particularly beneficial for subordinate individuals, who participate heavily in intergroup conflicts and intruder defense (Lazaro-Perea 2001, Radford 2008).

Because groups hold territories, the expectation is that groups will have long-term relationships with nearby neighbors, regardless of whether or not the neighbor is familiar. If individuals benefit from increasing their defense against predators when neighbors are present, there may be distinct advantages to exhibiting this behavior in the presence of unfamiliar neighbors. First, individual are more visually attentive to unfamiliar neighbors and monitor them at higher frequencies (Roberts 1988, Kutsukake 2006, 2007), possibly
due to the need to learn to recognize each other, judge competitive ability, and establish a social relationship (Stamps 1991). This means that a signal of cooperation or individual ability may be more likely to be received by unfamiliar neighbors. Second, there are also clear advantages of being familiar with neighboring groups (Bergmüller et al. 2005a). Since there is evidence that groups monitor predation events in neighbors (Heg et al. 2008a), playing a more active role in defense may increase the rate at which unfamiliar neighbors learn to recognize specific individuals (Dale et al. 2001). Finally, cooperating with neighbors, particularly unfamiliar neighbors with whom individuals have not yet established a cooperative relationship, may set a precedent for having a cooperative relationship in the future or preventing future conflict (Hamilton and Taborsky 2005a, Raihani et al. 2012).

In addition to direct benefits of intergroup interactions, there are alternative, but not mutually exclusive, hypotheses that may explain why subordinates increased their defense against the predator when neighbors were present. Subordinates compete with dominants for resources and reproductive opportunities (Buston 2003, Wong and Balshine 2011a), but mitigate these costs by helping to raise offspring, maintain the territory, and defend against intruders (Taborsky and Limberger 1981, Taborsky 1984, Balshine-Earn et al. 1998). Biological market theory predicts that subordinates should be highly valued and more tolerated when there are only a few (Noë and Hammerstein 1994, Kutsukake and Clutton-Brock 2008). The presence of neighboring groups increases the pool of potential subordinates and allows dominants to expect more help (i.e. more defense) in exchange for subordinate presence on the territory (pay-to-stay; (Wong and
Balshine 2011a). This is often accompanied by increased dominant aggression towards the subordinates in the presence of neighbors (Hamilton and Taborsky 2005b, Hellmann et al. 2015b). Although we found no evidence of this, subordinates can adjust their behavior in order to preemptively avoid dominant aggression, which may explain the lack of observable difference in aggression between treatments (Bergmüller and Taborsky 2005).

Social behavior is usually explored in the context of dyadic or within-group interactions, but the larger social environment is rarely accounted for when assessing individual decision-making. Our results demonstrate a specific influence of neighboring groups on individual behavior, but shifts in the social context are expected to alter intergroup dynamics. For instance, relatedness is expected to have a strong influence on intergroup interactions. Neighboring groups in the wild are often related to each other because individuals normally disperse to nearby territories (Stiver et al. 2007, Heg et al. 2008a). While neighboring groups in this experiment were unrelated to the focal group, there should be kin-selected benefits of cooperating with familiar relatives (Hamilton 1963) that are expected to influence the dynamics between neighboring groups. In addition, greater opportunities for extra-pair parentage and/or polygyny in a species may increase dominant response to neighboring groups. The fact that extra-pair paternity is more common than extra-pair maternity in this species (Dierkes et al. 1999) may explain why we found only a slight decrease in female aggression and no decrease in dominant male aggression when neighbors were present. Dominant individuals should be expected to reduce the amount they defend against the predator if subordinates willingly increase
their defense against the predator, but the fact that we did not see this may demonstrate that dominants receive smaller, but still significant benefits from neighboring groups. Exploring the influence of neighboring groups in a wider variety of social contexts is expected to further our understanding of the influence of intergroup interactions.

This study illustrates the importance of considering the effect of neighboring groups on individual behavior given that 1) intergroup dynamics can influence behaviors that do not directly involve conspecific interactions and 2) individuals of varying social statuses may have differential responses to neighbors. The presence of nearby groups offers direct opportunities to increase reproductive success through dispersal or extra-pair parentage. Individuals who can gain the most reproductive success from these opportunities (usually subordinates) should be most likely to respond to the presence of neighbors. However, these same individuals are often the most influenced by any change in intragroup dynamics that results from the presence of neighbors. It is likely that both of these mechanisms produce changes in individual behavior and future studies should attempt to separate the direct influence of neighbors from the accompanying change in intragroup dynamics in order to elucidate the relative influence of each on subordinate and dominant individuals. We suggest that further exploration into the relative influence of intragroup versus intergroup dynamics will provide important insight into the evolution of sociality by elucidating how the fitness benefits of social relationships change as a result of individual characteristics or the structure of the social environment.


Chapter 3: The Presence and Familiarity of Neighbors Influences Intragroup Network Dynamics and Sexual Conflict in a Group-living Fish

Abstract

Conflict is an inherent part of social life in group-living species. In many species, there is strong selection for group members to mediate aggression through the use of submissive and affiliative behaviors, which can stabilize the dominance hierarchy and foster group cohesion. However, the amount of conflict present in a group, as well as the way in which conflict is managed, likely varies with the presence of neighboring groups. Neighboring groups can promote intergroup conflict and incentivize group members to resolve or reduce intragroup conflict in order to facilitate cooperation in between-group conflict. We experimentally manipulated the visibility of neighbors in laboratory groups of *Neolamprologus pulcher*, a cooperatively breeding fish and recorded aggressive, affiliative, and submissive behavioral dynamics among dominant and subordinate group members when groups were isolated versus when neighbors were present. We then used exponential random graph models to understand how social network dynamics changed with the presence and familiarity of neighboring groups. We found that aggression and submission between mates was higher when groups were isolated, but that aggression between dominants and subordinates was relatively more frequent when groups had neighbors. This suggests that there is greater conflict between the dominant pair in the
absence of neighbors, but greater conflict between dominants and subordinates when neighbors are present. Additionally, we found that the dominant males maintained a prominent role in aggression networks across all time periods, but that dominant males play a less prominent role in affiliative intragroup networks when neighbors are present. This suggests that the initiators of affiliation within groups may change more plastically with respect to the social environment than initiators of within-group aggression. Collectively, these results provide some of the first empirical insights into the extent to which intragroup behavioral networks are mediated by intergroup interactions and the broader social context.
Introduction

Conflict is an inherent part of social life in group-living species, as group members frequently fight over the distribution of resources and reproduction. Conflict, which often manifests as aggression between group members, can negate the benefits of group living by increasing social stress, reducing group productivity and leading to group dissolution if left unresolved (Aureli et al. 2002, Young et al. 2006). In many group-living species, subordinate individuals make use of both submissive and affiliative displays to mitigate aggression from dominant group members and increase their likelihood of being tolerated within the group (Huntingford and Turner 1987, Bergmüller and Taborsky 2005). Submissive behavior can facilitate group stability by enforcing dominance hierarchies and expeditiously settling conflicts between individuals within a social group (Wilson 1975, Drews 1993), whereas affiliative behavior (e.g. grooming in primates) can reinforce friendships, promote reconciliation, and promote intragroup cooperation (Anacker and Beery 2013, Bruintjes et al. 2016).

The frequency of aggressive, submissive, and affiliative behaviors exchanged among group members often varies across groups (Kutsukake and Clutton-Brock 2008, Madden et al. 2009, Kutsukake and Clutton-Brock 2010), as well as across time within the same social group (Cantor et al. 2012, Godfrey et al. 2013, Bierbach et al. 2014). Given that social stability and the nature of social interactions within a group have important implications for individual fitness (Silk et al. 2003, Barocas et al. 2011), there have been empirical efforts to understand factors that modulate social dynamics within and across groups. Mounting evidence demonstrates that social interactions within a
group are correlated with group-level attributes, such as group size (Kutsukake and Clutton-Brock 2008, Madden et al. 2009, Kutsukake and Clutton-Brock 2010, Fischer et al. 2014, Shen et al. 2014), the relative size of group members (Hamilton et al. 2005), and the sex of group members (Kutsukake and Clutton-Brock 2008). However, much less is known about how the social environment beyond the level of the group, specifically the presence of other conspecific groups, influences social dynamics among group members.

There are two main ways in which the presence of neighboring groups may alter within-group social dynamics. First, neighboring groups can threaten the territory or resources of an established group and may incentivize group members to quickly resolve or reduce conflict within their own group in order to facilitate cooperation in between-group conflict (Radford 2008, Bruintjes et al. 2016). Studies examining territorial intrusions and conflict have found that affiliation between dominant and subordinate group members is greater when intergroup conflict is higher (Radford 2008, Bruintjes et al. 2016). In these cases, affiliative behavior is likely exchanged for continued participation in out-group conflicts (Seyfarth and Cheney 1984, Barrett et al. 1999, Radford 2011). Second, neighboring groups offer opportunities for dominants to replace current subordinates. The presence of neighbors provides a pool of subordinates that can potentially join the group; biological market theory predicts that this possibility of exerting partner choice may make dominants less tolerant of current subordinates, resulting in increased aggression toward subordinate group members when neighbors are present (Noë and Hammerstein 1994, Hellmann et al. 2015b, Hammerstein and Noë 2016). This suggests that neighboring groups can alter within-group dynamics and that
the familiarity of neighbors may determine the way in which neighboring groups alter intragroup interactions. When novel neighboring groups present threats to a group’s resources, intragroup conflict may be reduced temporarily; however, the initial reduction in intragroup conflict due to increased intergroup conflict may not persist as the presence of established neighbors may threaten group stability and foster conflict between group members due to dominant ability to replace evicted subordinates.

We experimentally manipulated the visibility of neighboring groups to understand how the presence and familiarity of neighboring groups was associated with changes in within-group social dynamics. To do this, we experimentally created 31 groups of *Neolamprologus pulcher*, a cooperatively breeding cichlid fish native to Lake Tanganyika in East Africa. These fish form colonies of 2-200 permanent territorial groups (Stiver et al. 2007). Each group is composed of a dominant male and female pair, with 1-20 subordinates that provide help in the form of territory defense, territory maintenance, and alloparental care (Wong and Balshine 2011a). Dominance is strictly size-based, such that the dominant male and female are the largest individuals and subordinates form sex-specific, size-based dominance hierarchies in which the largest male and female subordinates are most likely to inherit the dominant position (Wong and Balshine 2011a). Male group members maintain consistent differences in size, which likely reduces conflict that would otherwise arise among similarly sized individuals (Heg et al. 2004b, Hamilton et al. 2005, Hamilton and Heg 2008). Submission has been shown to be an effective appeasement behavior to reduce aggression in *N. pulcher* (Bergmüller and Taborsky 2005, Bruintjes and Taborsky 2008, Riebli et al. 2011, Hick et al. 2014),
while affiliative behavior is used to reinforce participation in territory defense (Bruintjes et al. 2016) and is associated with reduced cortisol levels (Ligocki et al. 2015b).

After isolated groups stabilized following group formation, we exposed groups to novel neighbors and observed behavioral interactions within and between groups when neighbors were novel and then later when neighbors had been visible for 30 days. We predicted that aggression between groups would be highest when neighbors were novel, as conflict between groups is highest when territory boundaries have not yet been established. We predicted that intragroup submission and aggression would be highest when groups were isolated and lowest when groups had novel neighbors, as conflict among group members is likely highest when there is no intergroup conflict to dilute intragroup conflict and lowest when there is the greatest amount of intergroup conflict (Radford 2008, 2011). In contrast, we predicted that affiliative behavior would be lowest when groups were isolated and highest when neighbors were novel, as affiliative behavior can be used to promote cooperation among current group members, especially when potential for intergroup conflict is high (Radford 2008, 2011).

**Methods**

**Housing conditions.** During the experiment, two groups were placed on opposite sides of a barrier in a 208-L (122cm long x 32cm wide x 53cm high) aquaria lined with 3cm of black sand substrate. Each group had two inverted terracotta flowerpot halves that served as shelter and potential breeding substrate, as well as two PVC tubes near the top of the tank that served as hiding spots for subordinate fish. To mirror natural conditions, a
12:12h light: dark schedule was maintained for the duration of the experiment and water temperatures were kept constant at 27°C ±1°C. Fish were fed daily and ad libitum with either TetraMin flakes (5 days a week) or frozen Daphnia or Artemia (twice per week). All experimental fish were wild caught or F1 offspring of wild-caught fish from the Kambwimba region of Lake Tanganyika. Prior to the experiment, all fish were marked uniquely with elastomer dye and given a dorsal fin clip to indicate sex. Fish recover from this procedure rapidly and receiving these markers has no apparent effect on subsequent behavior (Stiver et al. 2004, Dey et al. 2015). On the day that groups were formed, all group members were weighed to the nearest 0.001g and measured to the nearest 0.01mm.

The two groups in each aquarium were separated by two clear plexiglass barriers that were flush against the walls and floor of the aquarium and extended above the top of the water line. This largely prevented water flow between the two groups, although some water flow between the groups was possible and therefore, it is possible that chemical communication between the groups occurred. During the first 40 days after group formation, there was an opaque barrier between the plexiglass barriers that prevented groups from seeing or interacting with each other (Figure 4). After 40 days, the opaque barrier was removed and groups were able to interact across a clear barrier (Figure 4). All eggs that were laid during the experiment were removed immediately from the groups to remove any confounding influence of parental care on intragroup and intergroup network dynamics, although a previous study in this species found that the structure of interaction networks does not strongly vary with reproductive events (Dey et al. 2015). Nevertheless,
any behavioral observations conducted within 24hrs of egg-laying and removal were removed from the dataset.

**Group formation and behavioral observations.** From October 2014 to November 2015, we formed 31 social group of unrelated *N. pulcher*, each composed of a dominant male and female breeding pair and up to 3 subordinates. In these groups, the dominant male was the largest (and therefore, more dominant) fish in the group, followed by the dominant female; subordinates were all at least 5mm shorter in SL than dominants. We assembled up to 8 groups at a time; we formed new groups by reshuffling former group members or using new individuals so that each individual (n=99) used in this experiment was a member of two social groups throughout the course of the 13-month experiment. All individuals were reproductively mature (SL >35mm). All subordinates were unrelated to the dominants within their group, and all group members had never interacted prior to group formation. Groups were given 30 days after group formation to stabilize, as aggression can be high while dominance hierarchies are being established. In the initial days after group formation, some subordinates received high levels of aggression from the dominants and were subsequently removed from the group to prevent further injury. Consequently, at 30 days post-group formation, we had 11 social units that were composed only of a male and female pair and 20 social units that were composed of a dominant male and female pair with 1-3 subordinates. This range of group size allowed us to test if group composition impacts how group dynamics change in response to the presence and familiarity of neighbors.
At 30-days post formation, pairs and groups were observed for 30 minutes daily for 10 days. Observations during this period were highly correlated, suggesting that group dynamics had stabilized by Day 30 (Pearson’s correlation, Day 30 vs. Day 39:
Aggression: $t_{114}=4.30$, $p<0.001$, $r=0.37$; Submission: $t_{114}=3.74$, $p<0.001$, $r=0.33$,
Affiliation: $t_{114}=15.24$, $p<0.001$, $r=0.82$). After ten days, the opaque barrier isolating the groups was removed, such that groups were now visible to their neighbor on the other side of the tank. Groups were observed for 30 minutes daily for the first 10 days after removal of the opaque barrier to understand the impact of unfamiliar neighbors on intragroup dynamics (Days 40-49; Figure 1). Thirty days after the removal of the opaque barrier, we observed groups again for 30 minutes daily for 10 days (Days 70-79; Figure 1). All behavioral observations were recorded and videos were scored by the same observer (JKH) using species-specific ethograms (Sopinka et al. 2009, Ligocki et al. 2015a, Reddon et al. 2015). Behaviors were categorized as overt aggressive attacks (ram, bite, mouth fight), restrained aggressive displays (fin raise, fast approach, operculum spread, head jerk, head down display), submissive displays (tail quivers, hook, submissive posture), and affiliative behaviors (parallel swim, bump, join).
Figure 4: Diagram of experimental set-up dictating observation periods (Days 30-39, Days 40-49, and Days 70-79).

Statistical and network analysis. We used Wilcoxon rank-sum tests to determine differences in group aggression towards neighbors when neighbors were novel (Days 40-49) and when neighbors were established (Days 70-79). We also used generalized linear models (GLMs: R package MASS) with a negative binomial distribution and Tukey’s honest significant difference tests (R package multcomp) to test for differences in aggression towards neighbors due to individual status (dominant males, dominant females, subordinates).

To test how affiliative, submissive, and aggressive interactions between the dominant male and female pair changed with the presence and familiarity of neighbors, we used generalized linear mixed models (GLMM) with a negative binomial distribution because count data were overdispersed (R package glmmADMB). Dependent variables were the sum of all affiliative, submissive, or aggressive behaviors observed across the 10 observations. In each model, we included fixed effects of treatment (isolated, Days 30-
39; new neighbors, Days 40-49; established neighbors, Days 70-79) and the presence of male and female subordinates in the group, to control for variation in mate behavior due to group composition. We also included a fixed effect of the amount of aggression received from their mate, to account for variation in behavior due to their mate’s activity. For models examining affiliative behavior, we also included the fixed effect of the amount of affiliation received from their mate. To account for repeated measures, we included the random factor of group identity in models in which we compared multiple treatments (e.g. Days 40-49 to Day 70-79). We also included random factors of individual ID in all models, because each individual was in two groups.

To quantify how within-group social dynamics change with the presence and familiarity of neighboring groups, we analyzed social network structure in the 20 groups with a dominant pair and subordinates (R version packages statnet (Handcock et al. 2008), ergm (Hunter et al. 2008), and ergm.count (Krivitsky 2013)). We built weighted, directed networks of aggression, submission, and affiliation for each time period (isolated: Days 30-39; new neighbors: Days 40-49; established neighbors: Day 70-79). The weight of the edges in these networks was determined by the total number of interactions directed from one individual to another across the 10 observations in each time period. In addition to considering separate models of each time period, we also tested differences in the network structure between time periods by assembling a difference network. This network had the same set of nodes as the networks for each time period, but each edge weight was the difference in the number of interactions between two time periods (i.e. between Days 30-39 and Days 40-49, between Days 30-39 and Days 70-79, and between
Days 40-49 and Days 70-79). Because all edges must be positive values, we added the absolute value of the minimum edge weight to all within-group edge weights to make all values positive. Therefore, higher values denote that behaviors were more common in the later period (e.g. Day 40-49) than the earlier period (e.g. Day 30-39).

We analyzed network structure using exponential random graph models (ERGMs). As in logistic regression, these models test how independent variables predict the weight of the edges. Similar to Dey et al. (2015), we assembled a supernetwork composed of all 20 social groups for each time period and restricted all possible edges to those occurring among group members. To examine factors that contribute significantly to determining network structure, we tested the independent variables of: 1) the ‘sum’ term, which is equivalent to the intercept term in a regression model, 2) status homophily, which tests if there an increased chance of interactions between individuals of the same status (dominant or subordinate), 3) sexual homophily, which tests if there an increased chance of interactions between same-sex dyads, 4) actor effect of sex, which tests if one sex is more likely to initiate behavioral interactions, and 5) dyadic differences in size (SL), which tests if interactions are more likely to occur between individuals close in size. Further, we tested for structural dependence between edges by evaluating the tendency for 6) reciprocity, which tests if the weight of an edge from one group member to another predicts the weight of the reciprocal edge, and 7) cyclical triads, or the tendency of individuals to form cyclical triads, which are markers of unstable dominance hierarchies. The models use a Markov-chain Monte Carlo (MCMC) estimation technique to approximate the maximum likelihood and we specified a chain length of ten million, a
sampling interval of five thousand and a burn-in of fifty thousand proposals. We used Poisson reference graphs for each model (Dey et al. 2015) and models were visually checked for degeneracy and goodness of fit using the mcmc.diagnostics function. Examinations of model diagnostics did not indicate a high correlation between status homophily and size difference; therefore, we included both terms in our ERGMs.

**Results**

**Between-group interactions.** In general, group members were more aggressive to neighbors when they were initially exposed to neighbors (Days 40-49) compared to when neighbors had been present for 30 days (Days 70-79; Wilcox rank sum test: $W=5268$, $p=0.02$; Figure 5). When groups were initially exposed to neighbors (Days 40-49), dominant males were more aggressive to neighbors than were dominant females (GLM with negative binomial distribution and Tukey’s HSD: $Z_{91}=2.50$, $p=0.03$) and both dominant males and females were more aggressive to neighbors than were subordinates (DM: $Z_{91}=-7.07$, $p<0.001$; DF: $Z_{91}=-9.57$, $p<0.001$). However, when neighbors were established (Days 70-79), dominant females and dominant males showed similar levels of aggression ($Z_{91}=1.14$, $p=0.49$; Figure 5), although both were still more aggressive to neighbors than were subordinates (DM: $Z_{91}=-4.12$, $p<0.001$; DF: $Z_{91}=-5.26$, $p<0.001$). Dominants were also more aggressive to neighbors when there were no subordinates present in their group (i.e. when the social unit was composed of only a dominant male and female pair) compared to when there were subordinates in their social unit ($Z_{24}=-2.47$, $p=0.01$).
Are interactions between mates mediated by the presence and familiarity of neighbors?

Dominant males were significantly less aggressive to dominant females when neighbors were present (Days 40-49) compared to when they were absent (Days 30-39; Table 1; Figure 5). Dominant females were also less aggressive to dominant males when neighbors were present ($Z_{26}=-2.49$, $p=0.01$), but this trend did not persist when controlling for variation in the amount of aggression received from the dominant male (Table 1; Figure 5). Dominant males who were more aggressive to neighbors were also more aggressive to their mate ($Z_{22}=2.50$, $p=0.01$), but aggression to neighbors and aggression to mates were not significantly correlated for dominant females ($Z_{24}=1.06$, $p=0.29$). However, dominant females were more aggressive to dominant males when there were subordinate females in the group (Table 1).

Affiliative behavior between mates did not vary with the presence or absence of neighbors (Table 1; Figure 6), but dominant females were less affiliative to dominant males when there were subordinate males in the group (Table 1). Dominant females were significantly more submissive to dominant males when groups were isolated (Days 30-39) compared to when groups had neighbors (Days 40-49), even after controlling for variation in the amount of aggression received from the dominant male (Table 1; Figure 6). Dominant females who were more aggressive to their neighbors also demonstrated fewer submissive displays to dominant males, even after controlling for variation in the amount of aggression received by the dominant male ($Z_{21}=-2.43$, $p=0.02$).
There were no differences in the amount of aggression, affiliation, or submission exchanged between the dominant male and female between when neighbors were novel (Days 40-49) versus when neighbors were established (Days 70-79; GLMM; Aggression: $Z_{22}=-0.93$, $p=0.35$; Affiliation: $Z_{22}=-1.23$, $p=0.22$; Submission: $Z_{22}=0.25$, $p=0.80$; Figure 5-6).

_Are interactions within a group mediated by the presence and familiarity of neighbors?_ Aggressive, submissive, and affiliative networks were characterized by a strong, negative effect of reciprocity and cyclical triads across all time periods (Table 2), indicating that dominance hierarchies were stable across all time periods.

Across all time periods, males were more aggressive than females (sex: Table 2). Males were consistently more aggressive to other males than to females (sexual homophily: Table 2), although males were more sexually homophilic when neighbors were established compared to when they were new (positive effect of sexual homophily: Table 3). Females were similarly aggressive to males and females when groups were isolated and when neighbors were established, but more aggressive to males than to females when neighbors were novel (Table 2).

When groups were isolated, aggression was frequent between group members with a large size difference (positive effect of SL difference: Table 2) and dominant aggression was more frequently directed to mates rather than to subordinates (dominant status homophily: Table 2). In contrast, when neighbors were present, aggression was most frequently exchanged between individuals that were similar in size (Table 2) and
dominant aggression was more frequently directed to subordinates rather than to their mate. These changes in aggression with regard to size and status contributed significantly to changes in network structure between isolated groups and groups with neighbors (Table 3).

Females were more submissive than males when groups were isolated and when groups had new neighbors (actor sex: Table 2), although a significant effect of actor sex in the difference network indicates that this trend was stronger when groups were isolated compared to when groups had neighbors (Table 3). While males were equally submissive to males and females when groups were isolated, as well as when groups had novel neighbors, males were significantly more submissive to other males when groups had novel neighbors (sexual homophily: Table 2). In contrast, females were much more submissive to males than to females when groups were isolated, but that trend was significantly weaker when groups had neighbors (Table 3), especially when neighbors were familiar (Table 2). Subordinates were consistently more submissive to dominants than they were to other subordinates, although this did not reach significance for groups with established neighbors (Table 2). When groups were isolated and when groups had established neighbors, submission was most frequently exchanged between group members with a large size difference (SL difference: Table 2). However, when groups had novel neighbors, size did not significantly impact submission between group members (SL difference: Table 2).

Females were more affiliative than males across all time periods (actor sex: Table 2), but this effect was stronger when neighbors were present compared to when neighbors
were absent and contributed significantly to changes in network structure between the time periods (Table 3). Across all observation periods, males were more affiliative with females than males and females were more affiliative with males than females (sexual homophily: Table 2). Similarly, dominants were consistently more affiliative to their mate than to subordinates and subordinates were consistently more affiliative to other subordinates than to dominants (Table 2). Across all time periods, affiliative behavior was more likely to occur between individuals with a large size difference (SL; Table 2); however, this effect was stronger when neighbors were established compared to when groups were isolated and when groups had novel neighbors (Table 3).

**Table 1**: Results of generalized linear mixed models (negative binomial distribution) testing the effect of treatment (isolated or neighbors), the number of subordinates in the group, aggression received and affiliative behavior received on behavioral interactions between the dominant male and female pair.

<table>
<thead>
<tr>
<th>Isolated versus new neighbors</th>
<th>Dominant male to dominant female</th>
<th></th>
<th></th>
<th>Dominant female to dominant male</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aggression</td>
<td>Affiliative</td>
<td>Aggression</td>
<td>Affiliative</td>
</tr>
<tr>
<td></td>
<td>$Z_{24}$</td>
<td>$P$</td>
<td>$Z_{24}$</td>
<td>$P$</td>
</tr>
<tr>
<td>Treatment</td>
<td>-2.85</td>
<td><strong>0.004</strong></td>
<td>1.88</td>
<td>0.06</td>
</tr>
<tr>
<td>Sub. male</td>
<td>-0.52</td>
<td>0.60</td>
<td>0.33</td>
<td>0.74</td>
</tr>
<tr>
<td>Sub. female</td>
<td>-0.78</td>
<td>0.44</td>
<td>1.03</td>
<td>0.30</td>
</tr>
<tr>
<td>Agg. received</td>
<td>3.21</td>
<td><strong>0.001</strong></td>
<td>-0.41</td>
<td>0.68</td>
</tr>
<tr>
<td>Aff. received</td>
<td>—</td>
<td>—</td>
<td>4.19</td>
<td>&lt;<strong>0.001</strong></td>
</tr>
</tbody>
</table>

41
Table 2: Results of ERGM fit for behavioral networks for isolated groups (Days 30-39), groups with new neighbors (Days 40-49), and groups with established neighbors (Days 70-79). Although model fits for submission were good, cyclical triads could not be calculated for Days 40-49 and Days 70-79 because networks were sparse.

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>Aggression</th>
<th>Submission</th>
<th>Affiliation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Estimate</td>
<td>SE</td>
<td>P</td>
</tr>
<tr>
<td>Sum</td>
<td>1.74</td>
<td>0.07</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Difference in SL</td>
<td>0.07</td>
<td>0.003</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sexual homophily</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male-to-male</td>
<td>0.33</td>
<td>0.05</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fem-to-fem</td>
<td>0.06</td>
<td>0.05</td>
<td>0.19</td>
</tr>
<tr>
<td>Actor sex (male)</td>
<td>0.12</td>
<td>0.04</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Status homophily</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dom-to-dom</td>
<td>0.37</td>
<td>0.05</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sub-to-Sub</td>
<td>-0.86</td>
<td>0.06</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cyclical triads</td>
<td>-0.70</td>
<td>0.05</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Reciprocity</td>
<td>-1.16</td>
<td>0.03</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NEW NEIGHBORS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sum</td>
<td>2.77</td>
<td>0.07</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Difference in SL</td>
<td>-0.04</td>
<td>0.003</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sexual homophily</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male-to-male</td>
<td>0.54</td>
<td>0.05</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fem-to-fem</td>
<td>-0.16</td>
<td>0.06</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Actor sex (male)</td>
<td>0.12</td>
<td>0.05</td>
<td>0.01</td>
</tr>
<tr>
<td>Status homophily</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dom-to-dom</td>
<td>-0.36</td>
<td>0.05</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sub-to-Sub</td>
<td>-1.27</td>
<td>0.07</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cyclical triads</td>
<td>-1.08</td>
<td>0.04</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Reciprocity</td>
<td>-1.08</td>
<td>0.05</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ESTABLISHED NEIGHBORS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sum</td>
<td>2.42</td>
<td>0.09</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Difference in SL</td>
<td>-0.02</td>
<td>0.004</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sexual homophily</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male-to-male</td>
<td>0.19</td>
<td>0.06</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fem-to-fem</td>
<td>-0.04</td>
<td>0.06</td>
<td>0.56</td>
</tr>
<tr>
<td>Actor sex (male)</td>
<td>0.43</td>
<td>0.06</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Status homophily</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dom-to-dom</td>
<td>-0.52</td>
<td>0.06</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sub-to-Sub</td>
<td>-1.14</td>
<td>0.08</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cyclical triads</td>
<td>-0.44</td>
<td>0.07</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Reciprocity</td>
<td>-1.37</td>
<td>0.06</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Table 3: Results of ERGM fit for difference networks comparing isolated groups (Days 30-39), groups with novel neighbors (Days 40-49), and groups with established neighbors (Days 70-79). Negative estimates indicate that behaviors occurred more frequently in the earlier treatment (listed first), while positive estimates indicate that behaviors occurred more frequently in later periods (listed second).

| ISOLATED VS NEW NEIGH | Aggression | | | Submission | | | | Affiliation | | |
|-----------------------|------------|-------|-----------|------------|-------|-----------|-------|-----------|-------|-----------|-------|
|                       | Estimate   | SE    | *P*       | Estimate   | SE    | *P*       | Estimate | SE    | *P*       | Estimate   | SE    | *P*       |
| Sum                   | 5.87       | 0.01  | *<0.001*  | 4.28       | 0.04  | *<0.001*  | 5.36     | 0.02  | *<0.001*  |
| Difference in SL      | -0.008     | 0.001 | *<0.001*  | -0.004     | 0.002 | *0.04*    | -0.001   | 0.001 | 0.20      |
| Sexual homophily      |            |       |           |            |       |           |          |       |           |          |       |
| Male-to-male          | -0.003     | 0.01  | 0.80      | -0.12      | 0.04  | *<0.001*  | -0.01    | 0.01  | 0.49      |
| Fem-to-fem            | -0.006     | 0.01  | 0.57      | 0.19       | 0.03  | *<0.001*  | -0.009   | 0.01  | 0.26      |
| Actor sex (male)      | -0.01      | 0.007 | 0.08      | 0.31       | 0.05  | *<0.001*  | -0.02    | 0.02  | *<0.001*  |
| Status homophily      |            |       |           |            |       |           |          |       |           |          |       |
| Dom-to-dom            | -0.05      | 0.01  | *<0.001*  | -0.13      | 0.03  | *<0.001*  | -0.001   | 0.01  | 0.88      |
| Sub-to-Sub            | -0.03      | 0.01  | *0.005*   | -0.03      | 0.03  | 0.29      | -0.007   | 0.01  | 0.58      |
| Cyclical triads       | -0.0008    | 0.005 | 0.85      | 0.098      | 0.02  | *<0.001*  | -0.05    | 0.005 | *<0.001*  |
| Reciprocity           | -0.04      | 0.005 | *<0.001*  | 0.13       | 0.03  | *<0.001*  | 0.002    | 0.007 | 0.82      |
| ISOLATED VS EST NEIGH |            |       |           |            |       |           |          |       |           |          |       |
| Sum                   | 5.58       | 0.03  | *<0.001*  | 4.36       | 0.06  | *<0.001*  | 5.02     | 0.03  | *<0.001*  |
| Difference in SL      | -0.01      | 0.002 | *<0.001*  | -0.004     | 0.003 | 0.26      | 0.009    | 0.002 | *<0.001*  |
| Sexual homophily      |            |       |           |            |       |           |          |       |           |          |       |
| Male-to-male          | 0.13       | 0.02  | *<0.001*  | -0.13      | 0.05  | *0.02*    | 0.009    | 0.03  | 0.75      |
| Fem-to-fem            | -0.06      | 0.02  | *<0.001*  | 0.17       | 0.05  | *<0.001*  | 0.005    | 0.02  | 0.82      |
| Actor sex (male)      | -0.006     | 0.007 | 0.42      | 0.29       | 0.07  | *<0.001*  | -0.06    | 0.02  | *0.001*   |
| Status homophily      |            |       |           |            |       |           |          |       |           |          |       |
| Dom-to-dom            | -0.16      | 0.03  | *<0.001*  | -0.11      | 0.05  | *0.02*    | 0.15     | 0.03  | *<0.001*  |
| Sub-to-Sub            | -0.06      | 0.02  | *0.005*   | -0.02      | 0.04  | 0.47      | 0.06     | 0.03  | *0.03*    |
| Cyclical triads       | -0.02      | 0.007 | *0.006*   | 0.04       | 0.02  | *0.05*    | 0.005    | 0.01  | 0.08      |
| Reciprocity           | -0.06      | 0.009 | *<0.001*  | 0.16       | 0.04  | *<0.001*  | 0.04     | 0.01  | *0.007*   |
| NEW NEIGH VS EST NEIGH|            |       |           |            |       |           |          |       |           |          |       |
| Sum                   | 5.58       | 0.02  | *<0.001*  | 3.04       | 0.08  | *<0.001*  | 5.46     | 0.03  | *<0.001*  |
| Difference in SL      | -0.02      | 0.002 | *<0.001*  | 0.02       | 0.005 | *<0.001*  | 0.006    | 0.001 | *<0.001*  |
| Sexual homophily      |            |       |           |            |       |           |          |       |           |          |       |
| Male-to-male          | 0.17       | 0.02  | *<0.001*  | -0.15      | 0.07  | *0.04*    | 0.19     | 0.02  | *<0.001*  |
| Fem-to-fem            | -0.02      | 0.02  | 0.25      | 0.005      | 0.05  | 0.93      | 0.01     | 0.02  | 0.58      |
| Actor sex (male)      | 0.007      | 0.01  | 0.53      | 0.06       | 0.05  | 0.22      | -0.05    | 0.02  | *0.006*   |
| Status homophily      |            |       |           |            |       |           |          |       |           |          |       |
| Dom-to-dom            | -0.09      | 0.02  | *<0.001*  | 0.17       | 0.07  | *0.008*   | -0.03    | 0.02  | 0.16      |
| Sub-to-Sub            | -0.11      | 0.02  | *<0.001*  | 0.09       | 0.06  | 0.12      | -0.03    | 0.02  | 0.18      |
| Cyclical triads       | 0.003      | 0.008 | 0.69      | 0.05       | 0.03  | 0.13      | -0.20    | 0.02  | *<0.001*  |
| Reciprocity           | -0.04      | 0.008 | *<0.001*  | 0.04       | 0.03  | 0.23      | 0.02     | 0.01  | 0.07      |

43
Figure 5: Dominant male and female aggression toward their mate, toward subordinates within their group, and toward the neighboring group for isolated groups (Days 30-39), groups with new neighbors (Days 40-49), and groups with established neighbors (Days 70-79)
**Figure 6:** Dominant male affiliation toward the dominant female (white), dominant female affiliation toward the dominant male (light gray), and dominant female submission toward the dominant male (dark gray) for isolated groups (Days 30-39), groups with new neighbors (Days 40-49), and groups with established neighbors (Days 70-79). Data presented are total counts of behaviors across the 10 observations in each treatment period, with 95% confidence intervals.
Figure 7: Aggressive supernetwork structure of for isolated *N. pulcher* groups (Days 30-39), groups with new neighbors (Days 40-49), and groups with established neighbors (Days 70-79). Node orientation was determined the Fruchterman-Reingold algorithm. Larger nodes represent dominant individuals, red nodes represent females, and blue nodes represent males. Thicker edges indicate that more aggression was exchanged between a given dyad.
Discussion

Here, we demonstrate that interactions between mates and among all group members vary with the presence and familiarity of neighboring groups. Aggression from the dominant male to the dominant female and submission from the dominant female to the dominant male were more frequent when groups were isolated compared to when groups had neighbors, suggesting that conflict between mates is higher in the absence of neighbors (Kutsukake and Clutton-Brock 2006). It is possible that the presence of neighbors dilutes aggression between mates by offering another outlet for individual aggression. Further, the presence of neighbors may dilute reproductive conflict between mates over the presence of subordinates. We found evidence of conflict between dominants due to the presence of subordinates in the group: dominant females were more aggressive to dominant males when there were subordinates females (i.e. reproductive competitors) in the group and less affiliative to dominant males when there were subordinate males (i.e. potential mates) in the group. Because neighbors are a more pressing threat to territory ownership and reproductive success than subordinates (O'Connor et al. 2015, Bruintjes et al. 2016), the presence of neighbors may reduce aggression between mates associated with reproductive conflict over subordinates.

While dominant females seemed to respond to aggressive dominant males via high levels of submission when groups were isolated, we suggest that dominant females use defense against neighbors as an appeasement tactic to manage conflict with the dominant male when neighbors are present. Other studies in cooperatively breeding species that have found that subordinate individuals use helping behaviors, such as
territory defense, in lieu of or in addition to submission and affiliation to appease more dominant group members (Balshine-Earn et al. 1998, Cockburn 1998, Bergmüller and Taborsky 2005, MacLeod et al. 2013). Consistent with these findings, we found that dominant females who were more aggressive to neighbors were less submissive (per aggressive act received) to their mate. This would suggest that the threat presented by neighboring groups reduces the amount of conflict between mates, as well as provides additional methods by which individuals can appease more dominant group members.

We found that aggressive, affiliative, and submissive networks for the groups as a whole varied with the presence of neighboring groups. Both the initiators and receivers of dominance interactions change when neighbors are present. First, while the dominant male maintained a prominent role in aggression networks both when neighbors were absent and present, they initiated less affiliation and therefore, played a less prominent role in affiliative networks, when neighbors were present compared to when they were absent. We suggest that when neighbors are present, dominant males maintain their role in intragroup conflict, but also invest heavily in between-group aggression, because neighboring males are a large threat to their dominance position (O'Connor et al. 2015). This would be consistent with the result of Desjardins et al. (2008), who found that dominant males defended most vigorously against intruder threats to their dominance position. It would also be consistent with our findings that dominant males were the primary participants in intergroup aggression. With males focused on both within-group and between-group aggression when neighbors are present, we suggest that this allows the dominant female to assume a more prominent role in regulating within-group social
dynamics that promote social cohesion. This conclusion is in agreement with literature in primates demonstrating that the initiators and frequency of affiliation (i.e. grooming) vary widely depending on the social environment within the group (Barrett et al. 1999, Barrett et al. 2002), but that group stability is dependent upon dominant males policing aggression within the group (Flack et al. 2006).

In addition to changes in the initiator of social interactions, the target of social interactions changed with the presence of neighbors. Specifically, dominant aggression is directed chiefly toward their mate when groups were isolated, but directed primarily toward subordinates when groups had neighbors. This suggests that dominants are generally less tolerant of subordinates when neighbors are present, which would be consistent with the results of previous studies in this species (Hellmann and Hamilton 2014, Hellmann et al. 2015). Neighboring groups offer opportunities for individuals to move between groups (Heg et al. 2008) and therefore, provide credibility to subordinate threats of leaving the group and dominant threats of eviction, as dominants can potentially replace evicted subordinates with subordinates in neighboring groups (Johnstone and Cant 1999). This possibility of partner choice has been shown to increase conflict among current partners in a variety of systems (Noë and Hammerstein 1994, Hammerstein and Noë 2016) and is expected to also influence the dynamics between dominants and subordinates in group-living species (Kutsukake and Clutton-Brock 2008).

After controlling for variation in social interactions due to status, we also found that aggression was exchanged primarily between group members with large size differences when groups were isolated, but exchanged chiefly between similarly-sized
group members when neighbors were present. In species with size-based dominance hierarchies such as *N. pulcher*, conflict tends to be greatest between similarly-size group members (Wong et al. 2007, Hamilton and Heg 2008, Heg and Hamilton 2008) because relative fighting ability is less certain between group members close in size (Reddon et al. 2011). It is possible that the presence of neighbors induces rank-related conflict due to the potential for new subordinates to join the group or current subordinates to leave the group. The joining of a new subordinate would benefit high-ranking group members, who would gain benefits from the additional help and protection associated with a greater number of subordinates (Heg et al. 2005, Ligocki et al. 2015a), but would be costly to low-ranking subordinates who would descend in the dominance hierarchy if a larger subordinate joined the group (Ligocki et al. 2015a). Similarly, the introduction of neighbors may disrupt or destabilize the dominance hierarchy within the group due to the potential for current subordinates to leave the group. Subordinate dispersal from the group can destabilize the dominance hierarchy and induce temporary aggression between group members of adjacent rank as group members re-establish their rank position (Wong and Balshine 2011b). It is possible that introducing the option for subordinates to leave the group may disrupt the dominance hierarchy in a similar way. Supporting this, we found that aggression among similarly-sized group members was greatest after initial introduction of neighbors and was still present, but relatively less frequent when neighbors were familiar. This suggests that the rank-related conflict induced by neighbors persisted, but decreased after group members initially reinforced their dominance position within the group. Collectively, these results suggest that dominance hierarchies
may be less stable in the presence of neighbors, who provide options for group composition to change more frequently than if groups were isolated and there were no opportunities for dispersal.

In contrast to previous studies (Radford 2008, 2011, Bruintjes et al. 2016), we did not find much evidence that affiliation between dominants and subordinates was associated with the potential for intergroup conflict. We observed little change in the frequency and target of aggression between isolated groups and groups with new neighbors. Bruintjes et al. (2016) found that post-conflict affiliation increased following experimental territorial intrusions by neighboring and non-neighboring conspecifics, which corroborated findings by Radford (2008, 2011) that affiliation is higher in green woodhoopoes following intergroup conflict. These studies both involved punctuated conflict with neighbors (or the potential for punctuated conflict), where neighbors intruded onto the focal group’s territory. This type of conflict is likely more intense than the constant nearby presence of neighboring groups, where territory boundaries were never crossed. Consequently, the intergroup conflict present in our study may have never presented a great enough threat to the focal group to promote higher within-group affiliation; however, further investigations into how out-group threats alter intragroup social dynamics would be highly useful for elucidating how within-group dynamics vary with the type and severity of out-group threat.

It is important to note that some water flow between groups was possible and therefore, chemical communication may have occurred between groups while groups were isolated. Therefore, differences in interactions when neighbors were blocked from
view versus visible may not be due so much to the complete absence of neighbors, but rather due to the absence of interactions with neighboring groups and differences in how much groups know about their neighbors (e.g. group size, size of group members). We also cannot completely eliminate the possibility that our observed differences between treatment periods are due to time, as observations when groups were isolated occurred closer to group formation than observations when groups had neighbors. However, we believe that this is unlikely given that we found greater changes in network dynamics between isolated groups and groups with new neighbors than between groups with new neighbors and groups with established neighbors, despite the fact that the latter comparison was separated by more time than the former comparison. Further, many of the strongest changes in network structure when groups were exposed to new neighbors persisted even when neighbors became familiar (e.g. status and sex homophily, size), suggesting that network dynamics changed greatly in response to the presence of neighbors, but then stabilized in that new configuration. However, further research comparing network structure in groups that form in the presence of neighbors versus those that form while isolated and are later exposed to neighbors would help distinguish the effects of time versus neighbors.

Dominance interactions among group members have strong fitness implications, dictating the survival, fecundity, and longevity of group members (Silk et al. 2003, Barocas et al. 2011). Therefore, studies elucidating how dominance interactions differ across time and space are critical for understanding variation in the benefits and costs of group-living, both between populations of the same species as well as between different
species. Here, we demonstrated that within-group dominance interactions vary plastically with the broader social context. Specifically, we found that conflict between mates is greater when groups are isolated, but that conflict between dominants and subordinates is relatively higher when neighbors are present. Further, rank-related conflict seems to increase in the presence of neighbors, particularly when neighbors were novel. This variation in the frequency and target of aggression is important to document, as a subordinate that faces frequent aggression from dominants is expected to behave very differently compared to a subordinate of the same rank who faces little aggression from dominants. However, this fine-scale variation in dominance interactions within animal groups has not been well-explored, often due to the difficulty of obtaining a sufficient number of groups to robustly test variables that contribute to network structure within a group. While groups formed in the laboratory likely have different behavioral dynamics than groups formed naturally in the wild, this experiment nevertheless demonstrates the power of ERGMs in detecting fine-scale changes in behavioral dynamics and providing deeper insights into the mechanisms underlying the structure of dominance interactions in animal societies.
Chapter 4: Reproductive Sharing in Relation to Group and Colony-level Attributes in a Cooperative Breeding Fish

Abstract: The degree to which group members share reproduction is dictated by both within-group (e.g. group size and composition) and between-group (e.g. density and position of neighbors) characteristics. While many studies have investigated reproductive patterns within social groups, few have simultaneously explored how within-group and between-group social structures influence these patterns. Here, we investigated how group size and composition, along with territory density and location within the colony, influenced parentage in 36 wild groups of a colonial, cooperatively breeding fish *Neolamprologus pulcher*. Dominant males sired 76% of offspring in their group, while dominant females mothered 82% of offspring in their group. Subordinate reproduction was frequent, occurring in 47% of sampled groups. Subordinate males gained more paternity in groups located in high-density areas and in groups with many subordinate males. Dominant males and females in large groups and in groups with many reproductively mature subordinates had higher rates of within-group parentage loss, but only at the colony edge. Our study provides the first comprehensive quantification of reproductive sharing among groups of wild *N. pulcher*, a model species for the study of cooperation and social behavior. Further, we demonstrate that the frequency of extra-pair parentage differs across small social and spatial scales.
Introduction

In group-living species, reproductive conflict can exist at multiple levels of the social structure. Group members may conflict over the distribution of reproduction within the group, as dominant individuals seek to monopolize reproduction while subordinate group members attempt to parasitize dominant reproduction, potentially facing punishment or eviction when doing so (Vehrencamp 1983b, Vehrencamp 1983a). Individuals within a group may also come into conflict when group members mate with neighboring individuals, as the offspring of neighbors may parasitize the resources and parental care of current group members (Griffin et al. 2013). Both within-group factors, such as group size and composition, and between-group factors, such as the density and location of neighboring groups, influence the ability of individuals to pursue reproduction within their group (Cohas et al. 2006, Heg et al. 2008b) as well as reproductive opportunities with neighboring individuals (Westneat and Sherman 1997, Møller and Ninni 1998). Therefore, it is difficult to understand variation in fitness or decision-making without exploring how both within-group and between-group social structure affect the quality and quantity of reproductive opportunities.

Within-group attributes can influence the degree of reproductive sharing within the group (reproductive skew: Vehrencamp 1983b, Vehrencamp 1983a). by altering the ability of dominant individuals to skew reproduction in favor of their own fitness interests. For example, dominants may be less effective at reproductively suppressing subordinate reproduction when there are many same-sex subordinates acting as reproductive competitors (Cohas et al. 2006, Heg et al. 2008b) or when these same-sex
subordinates are close in size to the dominant (Heg 2006). Furthermore, the frequency of extra-pair parentage in a group may be related to the size difference between the socially bonded male and female; males who are much larger than their socially bonded female may be better at preventing the female from reproducing with other males (Wagner et al. 1996) or females with larger, potentially higher quality males may be less inclined to pursue extra-pair matings (Møller and Ninni 1998).

Colony-level factors can also have a profound influence on reproductive dynamics. High densities of neighboring territories facilitate intergroup forays, providing females with opportunities to mate with neighboring males or to lay eggs in other female’s territories (Petrie and Kempenaers 1998). Further, competition with neighbors may be intense in densely inhabited areas and it may not always be possible to prevent one’s social mate from mating with a neighbor (Petrie and Kempenaers 1998). In addition to density, the spatial location of a territory relative to other territories may influence reproductive patterns. Territories on the edge of a colony tend to be more exposed to predators (Brown and Brown 1987, Forster and Phillips 2009). If individuals occupying these edge territories must perform more predator defense or practice more vigilance, they may have less time and energy available for reproductive activities (Neff et al. 2004). Additionally, because individuals from edge territories may be inferior competitors relative to individuals in the colony center (Coulson 1968), high quality individuals may exploit poor quality competitors and reproduce with their socially bonded mate (Morton et al. 1990). While it has been well-established that territory location can have a significant impact on the number of offspring produced (Sergio and Newton 2003,
Johnson 2007, Forster and Phillips 2009), far less is known about how the spatial location of a territory in relation to other territories influence extra-pair parentage.

Finally, within-group dynamics are not independent of the dispersal and reproductive opportunities offered by neighboring groups (Vehrencamp 1983b, Vehrencamp 1983a, Johnstone and Cant 1999). An individual’s ability to control its mate’s activity is likely reduced when there is an increased availability of high quality extra-pair mates in neighboring groups (Petrie and Kempenaers 1998, Johnstone and Cant 1999). Similarly, when there are a high number of opportunities to mate outside of the social group, dominants may be less concerned with suppressing subordinate reproduction within their own group. Further, if subordinates risk eviction to reproduce within the group, the willingness to engage in reproduction may rise if there are many groups to disperse to, or if the quality of their group is low relative to surrounding groups (Johnstone and Cant 1999, Buston and Zink 2009, Nonacs and Hager 2011). Hence, to better understand why and how patterns of reproductive sharing arise, within and between-group factors need to be considered in tandem.

To determine how between-group traits, within-group characteristics, and the interaction between these two levels of social structure impact rates of extra-pair parentage, we examined within-group and colony-level demographics in relation to parentage in 36 wild groups of *Neolamprologus pulcher*, a cooperatively breeding cichlid native to Lake Tanganyika, East Africa. These fish form colonies of 2-200 territorial groups, each with a dominant pair and 1-20 subordinate group members who help raise the offspring of the dominant pair (Wong and Balshine 2011a). Individuals primarily
interact with other members of their own social group and those belonging to neighboring social groups located within a 3m radius (Bergmüller et al. 2005a, Heg et al. 2008a). Individuals strongly prefer to settle on territories in the center of the colony and will often remain subordinates on center territories rather than become dominants on edge territories (Heg et al. 2008a). While there is physiological evidence of reproductive suppression in subordinates of both sexes (Fitzpatrick et al. 2006, Heg 2008), previous laboratory studies have shown that subordinate males and females can reproduce within their home groups (Heg 2008, Heg and Hamilton 2008, Heg et al. 2008b, Heg et al. 2009) and one study found evidence of subordinate female reproduction in the wild (Stiver et al. 2009). Here we investigated the possibility that dominants may lose parentage to neighbors, to subordinate group members, or to both.

We predicted that rates of extra-pair paternity (EPP) would be greater on the edge of the colony and at higher densities (Westneat and Sherman 1997, Møller and Ninni 1998). In contrast, we predicted that extra-pair maternity (EPM) would be low regardless of territory location or density, because females in this species can recognize and destroy competitor eggs (Heg and Hamilton 2008). Regarding within-group characteristics, we predicted that parentage loss would increase as group size and the number of same sex-subordinates increased (Cohas et al. 2006, Heg et al. 2008b) and that subordinates closer in size to the dominant would be more likely to reproduce (Heg et al. 2006). Finally, we predicted that a larger size difference between the dominant male and female would allow the dominant male to exert more control over group dynamics, facilitating
subordinate or neighboring female reproduction and suppressing subordinate or neighboring male reproduction (Wagner et al. 1996).

Methods

Study site and collection. Between February and April 2013, we sampled 36 *N. pulcher* groups found in Kasakalawe Bay, Lake Tanganyika, East Africa (8°46’ S; 31°46’ E) using SCUBA. Groups all had free-swimming offspring and were located in seven different colonies or subpopulations ranging in depth between 11 to 13.5 m. The sampled groups comprised less than 10% of the total number of groups in these colonies, in order to minimize disturbance in the colony. Colonies consisted of a clustering of distinct social groups each defending a territory. Colonies are separated from each other by large open expanses of sand and rocky rubble uninhabited by *N. pulcher* (Stiver et al. 2007, Wong and Balshine 2011a). Each group’s territory was defined as the area of rocks defended by the dominant male and female, who are the largest individuals in the group (Wong and Balshine 2011a). The dominant and subordinate fish were identified as part of the focal group if they swam repeatedly under the territory rocks (shelter) without eliciting aggression from other fish within the territory boundaries. We measured territory size for each focal group and mapped the distance to all conspecific neighbors within a 3m radius. A group was considered to be on the edge of the colony if half or more of that group’s territory bordered an unoccupied area, with no other conspecific group within 10m.

Using fence nets and hand nets, we captured all individuals in these 36 groups that were close to sexual maturity (>30mm standard length [SL]: the tip of the snout to
the base of the caudal fin (Taborsky 1984). We then injected 20 ml of a clove oil mixture (1 part clove oil; 3 parts 70% ethanol; 1 part water) into the brood chamber (a central rock used for breeding and shelter) to euthanize any free-swimming young (<10mm SL). Groups breed year round in this species, and young collected for this study were all estimated to be two to six weeks old. We brought all the collected fish to the surface, where we measured adult body mass (to nearest 0.001g) and body length (SL to nearest 0.01mm). Mature fish were euthanized by immersion in a lethal concentration of benzocaine and the gonads were removed from adults and weighed. The entire carcass of free-swimming young and a section of the dorsal fin from adult fish were preserved with 95% ethanol for parentage analysis.

**Parentage analysis.** We analyzed parentage in 36 groups (24 groups from the center and 12 groups from the edge of a colony) with a total of 397 offspring (mean: 11.0 ± 0.8 s.e. offspring per group). We genotyped adults and young using six loci previously used on this species (Heg et al. 2008b, Stiver et al. 2009): US783 (Schliewen et al. 2001); Tmo11, Tmo13, Tmo25 (Zardoya et al. 1996); LOC101 (Brandtman et al. 1999); UME003 (Parker and Kornfield 1996). We extracted genomic DNA from preserved fin clips and young (QIAGEN DNeasy Blood and Tissue Kits) and used it in multiplexed PCR reactions, which involved six primer pairs end-labeled with the fluorescent dyes (FAM, HEX and NED; QIAGEN Multiplex Kit). We amplified samples using a Eppendorf thermocycler with a touchdown program set to the following parameters: 15min at 95C; 8 cycles of 30s at 94C, 90s at 55C (decreasing 0.5C per cycle), 60s at 72C; 21 cycles of 30s
at 94°C, 90s at 50°C, 60s at 72°C; 30min at 60°C; 60s at 10°C. We ran amplified samples on an ABI DNA sequencer (3100 Genetic Analyzer) and scored the profiles using GeneMapper Analysis software.

We used CERVUS 3.0 to assign maternity and paternity at the 95% confidence level. The loci were highly variable with an average of 25 alleles per locus (see Appendix A: Supplementary Table 1). One locus (LOC101) significantly deviated from Hardy-Weinberg equilibrium due to a high number of null alleles and so we removed it from our analyses. The combined exclusion power of the remaining five loci averaged 0.998 for a single parent and over 0.9999 for a parent pair.

**Statistical analysis.** 98.2% of offspring genotyped had at least one assigned parent in the social group, indicating that both young and group members had been correctly identified and that, unlike some other cichlid species, this species does not appear to adopt unrelated fry (Sefc 2011). When two of our 36 focal groups were <3m apart, we tested adults from the other, nearby focal group as potential parents. When young could not be assigned to any collected potential father or mother, we determined the minimum number of mothers or fathers that had contributed to that group’s parentage by counting the number of alleles at each locus that did not come from the assigned mothers or fathers collected with the brood. As a conservative estimate, we assumed all unknown parents were heterozygotes and contributed two alleles per locus.

We examined the factors correlated with the number of offspring assigned to the dominant male/female within the sampled group and to the subordinates within the
sampled group (generalized linear mixed effects models (GLMER) with Poisson distribution; R version 3.0.2, glmmadmb package). We also tested factors influencing the number of parents contributing to a brood using cumulative link models (CLM) in the ordinal package. For all models, we tested the fixed effects of location within the colony (center versus edge), density of neighboring groups (number of conspecific groups within 3m), group size, number of same sex subordinates within the group, number of offspring collected (to control for variation in brood size), and relative size difference between the dominant pair ([Dominant male SL – Dominant Female SL]/Dominant Male SL). The colony of each focal group was included in GLMER models as a random effect, but could not be used in a cumulative link mixed model because the model would not converge. For all models, two-way interactions were tested and non-significant interactions were removed from the models. Models for males and females were run separately. None of the within-group characteristics (e.g. group size, dominant size) were significantly different between groups from the center versus edge of the colony, nor did they vary with the density of neighboring groups (Appendix A: Supplementary Table 2). Density also did not vary with location within the colony (LME: F_{1,28}=1.16, p=0.29).

It is possible that dominants were misidentified in the seven groups in which the dominants gained no parentage. In all other groups, dominants gained > 50% of parentage and it is unlikely they were misidentified. In one of the five groups where the dominant male gained no paternity, two extremely small subordinate males (30mm and 32mm SL) gained all paternity in that group. In two additional groups, extensive behavioral observations confirmed the identity of the dominant male. Therefore, we reran
our analyses excluding the other four groups with no dominant paternity (n=2) or maternity (n=2). Our results were largely robust to these exclusions (Appendix A: see Supplementary Tables 3,4) and therefore the full dataset is presented below.

**Ethical Note.** *N. pulcher* is neither endangered nor threatened. The physical territories were minimally disturbed during collection and new groups occupied the vacated territories within one to two days post-collection. All methods, including euthanasia techniques, were approved by the Zambian Department of Fisheries, the Ohio State University IACUC (protocol ID 2008A0095) and the Animal Research Ethics Board of McMaster University (Animal Utilization Protocol Number 10-11-71). Our procedures adhered to the guidelines of the Canadian Council for Animal Care, and the Animal Behavior Society.

**Results**

**Parentage loss.** Across our 36 study groups, dominant males fathered 76.3% of collected offspring (303/397 offspring genotyped). The dominant male fathered all offspring in 12 groups, the majority of offspring in 19 groups (mean paternity ± s.e: 72.8% ± 3.4), and no offspring in 5 groups. Of the 94 offspring that could not be assigned to the dominant male collected with the social group, 34 were assigned to a subordinate male in the same social group (mean 10.8% ± 3.7 s.e. of offspring within their social group), 4 were assigned to a subordinate male from another sampled group, 1 was assigned to a dominant male from another sampled group, and 55 could not be assigned to any male sampled (Fig. 8). These
unassigned offspring were likely fathered by males in groups that we did not sample or by males within the group that had been recently evicted or eaten by a predator. The number of fathers per brood ranged from 1-4 (mean: 2.1 ± 0.2 s.e.).

The dominant females mothered 81.6% of offspring (324/397 offspring genotyped). The dominant female collected with the focal group was the mother of all offspring in 18 of the social groups, the majority of offspring in 16 groups (mean maternity ± s.e: 75.2% ± 5.4), and no offspring in 2 groups. Of the 73 offspring not assigned to the dominant female, 23 were assigned to a subordinate female in the same group (mean 4.7% ± 1.6 s.e.), 2 were assigned to a dominant female from another sampled group, and 48 were not assigned to any female sampled (Fig. 8), meaning that these offspring were likely mothered by females in groups that we did not sample or by females within the group that were recently evicted or eaten by a predator. The number of mothers per brood ranged from 1-4 (mean: 1.6 ± 0.1 s.e.). The degree of maternity loss across social groups was not correlated to the rate of paternity loss (LME: F1,28=0.19, p=0.85).

Subordinate reproduction. Subordinate males reproduced in almost a third of the sampled groups (11/36) and subordinate females reproduced in a quarter of groups (9/36). Of the reproductively mature subordinates genotyped, 35% of male subordinates (13/37 males) and 19% of female subordinates (9/47 females) achieved some degree of reproductive success. Subordinate females as small as 36 mm SL reproduced and males as small as 30 mm SL reproduced, which for males is below the average size previously
reported for sexual maturation (Taborsky 1984). The likelihood of a male subordinate reproducing was not influenced by his body size relative to the dominant male ([SL Dominant – SL Subordinate]/SL Dominant; generalized linear models (GLM) with binomial error distribution: \(Z_{24}=0.95, p=0.34\)); however, female subordinates were more likely to reproduce if they were close in size to the dominant female (GLM: \(Z_{35}=-2.22, p=0.03\)). There was no influence of relative gonad size (gonad mass relative to body mass, GSI) on whether a subordinate of either sex reproduced (GLM: Males: \(Z_{24}=1.06, p=0.29\); Females: \(Z_{35}=-0.82, p=0.41\)).

**Group and colony level factors related to parentage loss.** Dominant males lost significantly more paternity and subordinate males gained significantly more paternity in larger groups, but this occurred only in territories on the edge of the colony (Table 4: Location by Group Size interaction). There was no strong influence of group size on dominant and subordinate paternity in groups in the center of the colony (Table 4). Subordinate males also gained more paternity in higher density areas and in groups with more males (Table 4). The number of fathers per brood increased with the density of neighboring groups and was higher on the edge of the colony compared to the center (Table 4). Groups in high-density areas and on the edge of the colony also had a higher number of offspring that could not be assigned to any collected male group member (GLMER; Density: \(Z_{29}=1.96, p=0.05\); Location: \(Z_{29}=2.97, p=0.003\), suggesting that neighboring males sired a larger proportion of offspring in those areas. Although gonadal investment (GSI) of dominant males did not vary with territory density, males on edge
territories had relatively larger gonads than males with territories in the center of the colony (LME; Location: F\textsubscript{1,33}=4.49, p=0.04; Density:F\textsubscript{1,33}=1.39, p=0.25).

On the edge of the colony, groups with many subordinate females had dominant females that lost more maternity, subordinate females that gained more maternity, and more mothers per brood compared to edge groups with fewer female subordinates (Fig. 9A; Table 5: Location by Female Subordinate interaction). In contrast, center groups with a high number of subordinate females had dominant females that retained more maternity, subordinate females that gained less maternity, and fewer mothers per brood compared to center groups with fewer female subordinates (Fig. 9B; Table 5). Interestingly, dominant females who were much smaller than the dominant male retained a larger portion of the maternity of their brood compared to dominant females who were close in size to the dominant male (Table 5). This result was driven by differences in dominant male size, as females paired with larger dominant males suffered less loss of maternity (Fig. 10; GLMM; Z\textsubscript{21}=2.05, p=0.04).
Table 4: Allocation of paternity within groups. Test statistics (with df) and p-values from full models measuring the effect estimates of location (center versus edge), density of neighboring groups, group size, relative size of dominant male to dominant female ([dominant male SL-dominant female SL]/dominant male SL), and the number of male subordinates on the number of offspring assigned to dominant and subordinate males within the sampled group (GLMER) and number of fathers contributing to the brood (CLM). Total number of offspring within each brood was included as a fixed effect to control for variation in brood size. Asterisks indicate significance, assessed at α=0.05.

<table>
<thead>
<tr>
<th>Predictors</th>
<th>Dominant male reproduction</th>
<th>Subordinate male reproduction</th>
<th>Number of fathers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( Z_{22} )</td>
<td>( P )</td>
<td>( Z_{22} )</td>
</tr>
<tr>
<td>Location</td>
<td>3.22</td>
<td>\textbf{0.001}</td>
<td>-2.92</td>
</tr>
<tr>
<td>Density of neighbors</td>
<td>-1.02</td>
<td>0.29</td>
<td>2.04</td>
</tr>
<tr>
<td>Group size</td>
<td>1.18</td>
<td>0.24</td>
<td>-1.89</td>
</tr>
<tr>
<td>Size difference</td>
<td>1.69</td>
<td>0.09</td>
<td>-1.28</td>
</tr>
<tr>
<td>Number of male subs</td>
<td>-0.39</td>
<td>0.70</td>
<td>3.24</td>
</tr>
<tr>
<td>Offspring</td>
<td>7.05</td>
<td>\textbf{&lt;0.001}</td>
<td>-1.65</td>
</tr>
<tr>
<td>Location * group size</td>
<td>-3.59</td>
<td>\textbf{&lt;0.001}</td>
<td>3.16</td>
</tr>
</tbody>
</table>
Table 5: Allocation of maternity within groups. Test statistics (with df) and p-values from full models measuring the effect estimates of location (center versus edge), density of neighboring groups, group size, relative size of dominant male to dominant female ([dominant male SL-dominant female SL]/dominant male SL), and the number of female subordinates on the number of offspring assigned to dominant and subordinate females within the sampled group (GLMER) and the total number of mothers contributing to the brood (CLM). Total number of offspring within each brood was included as a fixed effect to control for variation in brood size. Asterisk indicate significance, assessed at α=0.05.

<table>
<thead>
<tr>
<th>Predictors</th>
<th>Dominant female reproduction</th>
<th>Subordinate female reproduction</th>
<th>Number of mothers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$Z_{22}$</td>
<td>$P$</td>
<td>$Z_{22}$</td>
</tr>
<tr>
<td>Location</td>
<td>1.89</td>
<td>0.06</td>
<td>-2.85</td>
</tr>
<tr>
<td>Density of neighbors</td>
<td>0.29</td>
<td>0.77</td>
<td>0.87</td>
</tr>
<tr>
<td>Group size</td>
<td>-0.74</td>
<td>0.46</td>
<td>1.33</td>
</tr>
<tr>
<td>Size difference</td>
<td>1.96</td>
<td><strong>0.05</strong></td>
<td>-1.05</td>
</tr>
<tr>
<td>Number of female subs</td>
<td>1.89</td>
<td>0.06</td>
<td>-2.54</td>
</tr>
<tr>
<td>Offspring</td>
<td>4.70</td>
<td>&lt;0.001</td>
<td>4.16</td>
</tr>
<tr>
<td>Location * female subs</td>
<td>-2.41</td>
<td><strong>0.003</strong></td>
<td>3.46</td>
</tr>
</tbody>
</table>
Figure 8. The percent of offspring (pooled across all 36 sampled groups) belonging to the dominant male or female, (black) and to subordinate males and females in the social group (light grey), as well as all other instances of extra-group reproduction (in dark gray, a combination of reproduction assigned to known individuals outside the sampled group and reproduction that was unable to be assigned).
Figure 9. A) In the 12 groups on the edge of the colony, the number of offspring mothered by the dominant female decreased as the number of female subordinates in the group increased (GLMER: $Z_{6} = -2.97$, $p=0.003$). B) In contrast, in the 24 groups in the center of the colony, the number of offspring mothered by the dominant female increased as the number of female subordinates in the group increased (GLMER: $Z_{16} = 3.38$, $p<0.001$).
Figure 10. Across all 36 sampled groups, dominant females mothered more offspring (after controlling for variation in brood size) when they were mated with a large male, both in terms of absolute size (standard length [SL], as shown in this figure) and in terms of relative size in relation to the dominant female’s size (Table 5). Data presented are least squared means of the regression model testing predictors of the number of offspring mothered by the dominant female.
Discussion

Extra-pair paternity and extra-pair maternity were common in this study, accounting for 24% and 18% of the total number of sampled offspring respectively. For both males and females, extra-pair offspring were sired by subordinates within the group as well as by individuals from neighboring groups. Both within-group characteristics, including the size of the dominant male, and between-group characteristics, such as the density of neighboring groups, significantly influenced reproductive dynamics within groups. Further, the effect of some within-group characteristics, such as group size, varied with colony-level characteristics. Collectively, these results suggest that understanding of the reproductive dynamics within colonial groups requires comprehension of both within-group and between-group social dynamics.

This study provides strong evidence of extensive subordinate reproduction in wild groups of this well-studied fish species. We uncovered high levels of subordinate reproduction, with 35% of collected male subordinates and 19% of collected female subordinates achieving some degree of reproductive success. Previous studies based on much smaller samples sizes (Dierkes et al. 2008, Stiver et al. 2009) found no occurrences of subordinate male reproduction and only one occurrence of subordinate female reproduction in the wild, leading to the mistaken conclusion that subordinate reproduction is rare in free living *N. pulcher* groups.

High levels of subordinate reproduction in a cooperatively breeding system call into question current explanations for the fitness benefits of helping in these fish. If subordinates obtain high levels of reproduction, what is perceived to be subordinate
alloparental care may in fact represent direct parental care (Wong and Balshine 2011a). Further, if subordinates reproduce within their own groups, then lost reproductive opportunities resulting from delayed dispersal, considered to be one of the main costs of helping (Wong and Balshine 2011a), may have previously been overstated. Our study demonstrates that the evolution of apparent helping behavior in *N. pulcher* may be at least partly explained by direct reproductive benefits. Although some cooperatively breeding species have higher levels of subordinate reproduction than those reported in this species, this degree of subordinate reproduction is rare for cooperative breeders, as subordinates in many cooperatively breeding species are reproductively suppressed, either behaviorally or physiologically, by dominants (Russell 2004). In birds, the evolution of cooperative breeding is associated with lower rates of extra-group parentage (Boomsma 2007, Cornwallis et al. 2010), but cooperative breeding can be maintained even in highly promiscuous species if subordinates receive direct benefits while helping in the group (Richardson et al. 2002). Given the high level of extra-group parentage found in this study, some baseline subordinate reproduction may be necessary for assuring continued subordinate help and membership in the group (Bruintjes et al. 2011).

The social environment can also determine the level of within-group subordinate reproduction by altering the ability and motivation of subordinates to reproduce (Kokko and Ekman 2002, Nonacs and Hager 2011). On the colony edge, the portion of the brood mothered by subordinate females increased as the number of female subordinates within the group increased; however, in the colony center, groups with many female subordinates actually had comparatively less subordinate female reproduction. Similarly,
subordinate males reproduced more frequently in larger groups, but only on the edge of the colony. Compared to dominants in the colony center, dominants on the edge of the colony may be less capable of suppressing subordinate reproduction or they may concede more reproduction to subordinates because they are more reliant on subordinates for defense against predators (Bruintjes et al. 2011).

Alternatively, subordinates in central territories may choose not to reproduce, as getting evicted following reproduction (Dierkes et al. 1999) would result in losing the opportunity to inherit a high-quality territory in the center of the colony. This would be particularly costly for subordinates in large groups, which are more productive and more likely to persist from year to year (Heg et al. 2005, Awata et al. 2010). However, if there are lower fitness benefits to inheriting low quality territories on the colony edge (Heg et al. 2008a), the benefits of reproducing for subordinates may outweigh the costs of getting evicted due to reproduction. Similarly, subordinate males may reproduce more frequently in denser areas because eviction may be less costly when there are a high number of potential groups to join. Further, increased reproduction by neighboring males may mean that subordinates on the colony edge and in denser areas are less related to the offspring in their group compared to subordinates in the center or in less dense areas. Therefore, subordinates in the center or less dense areas may gain greater indirect fitness benefits, future fitness benefits, or both, by providing alloparental care, compared to subordinates on the edge or in denser areas.

In addition to high rates of subordinate reproduction, we found evidence of extra-pair maternity in about half of the sampled groups. Dominant females paired with large
males mothered a larger portion of offspring compared to dominant females paired with smaller males. Across a variety of taxa, larger and older males are higher quality mates, have more reproductive success, and are more likely to be extra-pair mates (Morton et al. 1990, Olsson and Shine 1996, Hsu et al. 2015). Dominant females paired with smaller males may be more likely to pursue mating opportunities with higher quality males in neighboring groups and spend less time guarding against reproductive competitors (Petrie and Kempenaers 1998). Large dominant males may also be more successful at cuckolding neighboring males and mating with females in neighboring territories. They may choose this strategy, rather than court females in their own territory, because reproduction of multiple females within a territory can increase within-group conflict (Young et al. 2006, Bell et al. 2014) and siring offspring on other territories allows males to parasitize the care and resources of other individuals (Griffin et al. 2013).

On the edge of the colony, a higher proportion of collected offspring were sired by males other than the collected dominant or subordinate males, suggesting that neighboring males steal more paternity from a given resident male on the edge of the colony compared to the center. Resident males on the edge of the colony may have a reduced ability to prevent other males from mating with their social mate, either due to decreased quality or to increased time spent vigilant against predators (Coulson 1968, Brown and Brown 1987, Neff et al. 2004, Forster and Phillips 2009); however, edge males may also adopt different mating strategies. In wild guppy populations (*Poecilia reticulata*), extra-pair paternity is much more common in high predation environments compared to low predation environments (Kelly et al. 1999). Experiments manipulating
predation pressure in guppies found that, under perceived high predation pressure, males reduce their courtship displays and sneak copulations more often (Godin 1995) while females become less selective regarding potential mates (Gong and Gibson 1996). This is likely because courtship displays and mating selection tend to attract predators (Sih 1994). This may also explain why males on the edge of the colony had the largest testes; edge males may invest substantially more in sneaking paternity in neighboring nests compared to males in the center.

Here, we provide support that both within-group and between-group factors are linked to reproductive patterns in N. pulcher. Further, we found that the influence of within-group characteristics may depend upon colony-level social structure. Our results demonstrate that studies of reproductive dynamics within groups should consider the influence of neighboring groups, which offer additional reproductive opportunities and can alter how individuals pursue reproductive opportunities within their own group. Our results suggest that individuals occupying lower quality edge territories may adopt different reproductive strategies to accommodate increased predation risk. Further, subordinates may attempt to reproduce when living in lower quality edge territories to compensate for lowered kin-selected benefits of alloparental care or the costs of living in a suboptimal habitat. We suggest that the patterns of extra-pair parentage and the selective pressures governing the pursuit of extra-pair parentage may vary over relatively small spatial and social scales. Further research exploring behavioral and reproductive exchanges within and between groups would be useful for elucidating the extent to which individuals vary their decisions based on the presence of neighboring groups.
Chapter 5: Genetic Relatedness Within Groups is Linked to Colony-level Social Structure and Parentage Patterns in a Social Cichlid Fish

Abstract

In group-living species, the degree of relatedness among group members often governs the extent of reproductive sharing, cooperation, and conflict within a group. Kinship among group members may be partly determined by the presence and location of neighboring groups, which provide dispersal or mating opportunities that can dilute kinship among current group members. Here we assessed how within-group relatedness varies with the density and position of neighboring social groups in Neolamprologus pulcher, a colonial and group-living cichlid fish. We used restriction site-associated DNA sequencing (RADseq) methods to generate thousands of polymorphic, homologous SNPs. Relative to microsatellite data, RADseq data provided much smaller confidence intervals around relatedness estimates. This allowed us to document novel patterns of relatedness in relation to colony-level social structure. First, the density of neighboring groups was negatively correlated with relatedness between subordinate and dominant females, but was not significantly correlated with relatedness between subordinate and dominant males. Second, subordinates at the colony edge were less related to dominant males in their group than were subordinates in the colony center, suggesting a shorter breeding
tenure for dominant males at the edge of the colony. Finally, subordinates who were closely related to their same-sex dominant were more likely to reproduce, supporting some restraint models of reproductive skew. Collectively, these results demonstrate that within-group relatedness is influenced by the broader social context, and variation between groups in the degree of reproductive sharing between dominants and subordinates can be explained by both patterns of relatedness and the nature of the social landscape.
Introduction

Relatedness between group members strongly influences social and reproductive dynamics (Hamilton 1963, Keller and Reeve 1994, Kokko et al. 2002b). Variation in the average degree of within-group relatedness maps onto species-level differences in cooperative tendencies (Cornwallis et al. 2010) and accounts for the evolution of allocare across phylogenetically distinct groups of species (Briga et al. 2012). Within species, theoretical models predict that groups with low levels of kinship will have increased within-group aggression (Johnstone and Cant 1999), reduced group stability (Johnstone and Cant 1999), and increased reproductive sharing among group members (Vehrencamp 1983b, Vehrencamp 1983a, Keller and Reeve 1994, Whittingham et al. 1997). Further, in cooperatively breeding species, low relatedness between dominant breeders and subordinate helpers is usually correlated with reduced allocare by subordinates (Griffin and West 2003, Schneider and Bilde 2008, Nam et al. 2010, although see Stiver et al. 2005 and Zöttl et al. 2013b for exceptions). Consequently, given that kinship among group members drives social and reproductive dynamics within groups, exploring factors that promote variation in within-group relatedness can help us better understand why group dynamics differ both within and across populations.

The social structure beyond the level of the single group, specifically the number and location of neighboring groups, likely contributes to variation in the degree of kinship among group members observed across groups. Within-group relatedness decreases with high levels of extra-pair reproduction (Cornwallis et al. 2010) and with high turnover in group membership via subordinate dispersal and joining of unrelated
immigrants (Dierkes et al. 2005). Both group turnover and extra-pair parentage are likely
dependent on the social organization above the level of the group. For instance, having
many close neighbors is often correlated with increased extra-pair parentage (Westneat
and Sherman 1997) and an increased ability of individuals to move between groups
(Russell and Rowley 1993, Doolan and Macdonald 1996, Heg et al. 2008a). Thus,
individuals living in areas with high group density may experience lower within-group
relatedness relative to groups in less dense areas, where between-group movement and
extra-pair mating may be more challenging. In addition to the density of neighboring
groups, the relative location of groups on the edge versus center of a colony can also
influence the patterns of within-group relatedness. In many colonial species, territories
on the edge of the colony are suboptimal because these groups suffer increased predation,
higher rates of mortality of current group members, and increased rates of extra-group
paternity (Brown and Brown 1987, Forster and Phillips 2009, Hellmann et al. 2015a). For
all of these reasons, we expect to see lower levels of kinship among group members at
the edge of the colony compared to groups in the center of the colony.

Because there is evidence to suggest that relatedness among group members is not
solely driven by forces within the group, we sought to understand how within-group
kinship is altered by colony-level social structure in Neolamprologus pulcher, a
cooperatively breeding cichlid fish native to Lake Tanganyika, East Africa. These fish
form territorial groups comprised of a dominant breeding pair and 1-20 subordinates that
form size-based dominance hierarchies (Wong and Balshine 2011a). Individual social
groups are located in colonies of 2-200 groups (Stiver et al. 2007). While subordinate
females often attain breeding status by inheriting their natal territory, subordinate males typically disperse to fill vacant breeding positions in other territories (Balshine-Earn et al. 1998, Stiver et al. 2007, Wong and Balshine 2011a). Relatedness varies widely between and among groups (Stiver et al. 2005), likely because N. pulcher social groups have high levels of extra-pair parentage (Hellmann et al. 2015a), frequent dispersal between groups (Stiver et al. 2007), and high rates of group member turnover (Dierkes et al. 2005). These characteristics make this species an ideal system for understanding how colony-level social structure promotes variation in relatedness among group members.

While microsatellite loci have often been used to assess within-population relatedness, techniques that generate genomic-scale datasets, such as restriction site-associated DNA sequencing (RADseq) methods, are increasingly being used to assess genetic variation between populations or species (Martin and Feinstein 2014, Rasic et al. 2014, Viricel et al. 2014). By identifying variation in SNPs adjacent to restriction enzyme sites, RADseq data yield thousands of polymorphic, homologous SNPs and allow for the rapid acquisition of high-resolution genomic data without requiring any previous information about the genome (Baird et al. 2008). These features suggest that this novel technique has great potential for fine-scale analyses of relatedness in behavioral ecological research. Here, we assess this potential by using RADseq data to explore how within-group relatedness varies in relation to the density of nearby social groups and to the location of a group on the colony edge versus center of the colony. We also investigate if within-group relatedness is linked to patterns of reproductive sharing observed in N. pulcher and compared relatedness values generated from RADseq data to
those generated from microsatellite data. These analyses will help shed light on the extent to which loci derived from RADseq data might improve the precision of within-population relatedness estimates.

We predicted that within-group relatedness would be lower in denser areas of the colony where subordinates can more easily move between groups (Heg et al. 2008a) and in groups on the edge of the colony where dominants often lose more parentage relative to dominants in the center of the colony (Hellmann et al. 2015a). These effects on relatedness were expected to be stronger for male than for female *N. pulcher* because vacant positions in the group are more likely to be filled by immigrants for males than for females (Stiver et al. 2006), breeder turnover is more frequent for males than for females (Dierkes et al. 2005, Stiver et al. 2007, Jungwirth et al. 2015a), and extra-pair paternity is more common than extra-pair maternity (Hellmann et al. 2015a). Finally, we predicted that rates of extra-pair parentage would be higher when the dominant male and female were more related, to reduce potential costs associated with inbreeding depression (Arct et al. 2015). However, we did not predict to see a similar relationship with subordinate reproduction, because subordinate reproduction is more highly constrained and a laboratory study in this species found that the degree of subordinate reproduction did not vary with relatedness to dominants (Bruintjes et al. 2011).

**Methods**

**Study site and field collection.** From February to April 2013, we collected tissue samples from wild *N. pulcher* groups in Kasakalawe Bay, Lake Tanganyika, East Africa (8°46’ S;
31°46’ E) using SCUBA. Groups were dispersed among 7 colonies at depths ranging from 11m to 13.5m. Each colony consisted of many (7 to ~200) distinct social groups each defending a discrete territory. Colonies were separated from each other by large open expanses of sand and rubble uninhabited by *N. pulcher* (Stiver et al. 2007). Each focal group was observed prior to sampling to identify dominant and subordinate group individuals. An individual was considered to belong to the focal group if it swam repeatedly under the rocks within the group’s territory without eliciting aggression from other fish in the territory. Within each focal group, dominant and subordinate *N. pulcher* were differentiated by size, as dominance is very strongly linked to body size in this species (Reddon et al. 2011, Dey et al. 2013) and the largest male and female *N. pulcher* are almost always the dominant pair (Wong and Balshine 2011a). Parentage analysis of fry from these groups confirmed that the dominant individuals were correctly identified and collected in conjunction with their home territory (Hellmann et al. 2015a). All conspecific neighbors within a 3m radius were mapped in relation to the sampled focal group. Groups were defined as being on the edge of the colony if unoccupied areas bordered half or more of the group’s territory (i.e. there were no conspecific groups within 10m on that side of the territory; (Hellmann et al. 2015a). For each social group, all sexually mature individuals, as well as those close to sexual maturity (>30mm standard length (SL): the length from the tip of the snout to the base of the caudal fin; (Taborsky 1985), were captured using fence nets and hand nets. Dominants and subordinates that had been captured were then taken to the surface in mesh bags where they were weighed to the nearest 0.001g and measured to the nearest 0.01mm SL.
were euthanized by immersion in a lethal concentration of benzocaine (ethyl p-aminobenzoate, 1.0 µg/ml) for use in other studies (Hellmann et al. 2016). Sections of the dorsal fin were also taken from all individuals to be used as a genetic sample for relatedness analysis for this study.

**RAD methods.** A total of 31 groups (20 center groups, 11 edge groups) with 171 individuals (31 dominant females, 30 dominant males, 51 subordinate females, 44 subordinate males, and 13 reproductively immature subordinates) were analyzed. While we collected more than 31 groups (Hellmann et al. 2015a, Hellmann et al. 2016), we limited the analyses to these 31 groups because 1) we were confident that all group members were collected from these groups, 2) they contained 2 or more reproductively mature subordinates, and 3) samples from these groups produced sufficiently high quality DNA for collecting RADseq data. Individually-barcoded RAD libraries were generated using the protocol of Sovic et al. (2016) with the following modifications. EcoRI and PstI high fidelity restriction enzymes (New England Biolabs) were used for digestion, and 250 ng of genomic DNA was digested for 90 min at 37°C and then heat-inactivated for 20 min at 80°C. Samples were quantified with qPCR prior to library amplification, and a minimum threshold of 1x10⁶ molecules was required for each sample to help reduce possible effects of low library complexity. Samples not meeting this threshold were re-prepped prior to sequencing, as preliminary data suggested that samples below this value showed relatively high levels of missing data. Samples were sequenced in
pooled libraries of up to 36 individuals and sequenced in single-end 50-bp runs on an Illumina HiSeq 2500.

**Bioinformatics methods.** Demultiplexing, quality filtering, locus assembly, and genotyping were performed with AftrRAD v4.1 (Sovic et al. 2015) using default parameters, with the exception that the ‘re’ argument was set to ‘TGCAG’ to match the use of the restriction enzyme PstI. The default parameters include a 90% mismatch allowance between alleles when assembling loci, a minimum read depth of 10 for each allele for genotyping, and a minimum Phred score of 20 for each base in order for reads to be retained for analysis. Levels of missing data were assessed for each sample, and three individuals were removed from the dataset due to relatively high levels of missing data that appeared to be associated with low sequencing coverage/depth. Reads were screened for the build-up of artifactual SNPs at the end of reads; any SNPs beyond position 35 (after removing barcode and restriction sites at the beginning of reads) were omitted in this dataset. In total, we identified and removed 2,453 paralogous loci from the dataset. We also identified 40,447 monomorphic loci, which were not analyzed further, and 14,101 polymorphic loci. Of the polymorphic loci, 2,250 loci were scored in 100% of the 168 individuals remaining in our dataset. These 2,250 loci were then used to assess relatedness.

**Statistical analysis.** Relatedness was assessed using the package Related (Pew et al. 2015), which implements the code for COANCESTRY (Wang 2002) in R. We used the
measure of relatedness described by Wang (2002) because it is more accurate (minimizes root-mean standard error) than the measures of relatedness described by Queller and Goodnight (1989), Lynch and Ritland (1999), and Milligan (2003) (Wang 2011). It also better accounts for biases often associated with small sample sizes and samples that include clusters of relatives (i.e. groups of parents and offspring), which are characteristics of this dataset (Konovalov and Heg 2008). To further minimize potential biases arising from population structure, the dataset was split into 4 subpopulations, or grouping of colonies located in close proximity to each other (see Figure 11) and relatedness among group members was analyzed relative to the subpopulation (Wang 2011).

We used cumulative link mixed models (CLMM) to test predictors of a given subordinate’s relatedness to the dominant male and female in its group (R package ‘ordinal’; (Christensen 2012). All relatedness values were categorized as 0 (or below 0), 0.125, 0.25, and 0.5. We analyzed relatedness values as discrete values rather than continuous for two reasons: first, R values between -0.5 and 0 hold similar biological significance for the purposes of our hypotheses, and second, the data were not normally distributed, and a normal distribution could not be achieved through data transformation. Values were categorized by evaluating 95% confidence intervals around the point estimates of relatedness and placing data points into the category (0,0.125,0.25, or 0.5) that was within the confidence interval. For all data points, confidence intervals were small enough that they did not span more than one category. In one case, the calculated confidence interval did not encompass any of the categories (was between 0.25 and 0.5,
but not inclusive of either value), and so we conservatively assigned this data point in the 0.25 category. In the models, we tested independent variables describing the spatial location of the group (categorical: center or edge of the colony), the density of neighboring groups (continuous: the number of neighboring groups within a 3m radius), subordinate sex, and relative size of the dominant and subordinate \([(\text{dominant SL} – \text{subordinate SL})/\text{dominant SL})\]. The focal group of the subordinate (nested within colony) and the colony of the focal group were both included in the model as random effects. We chose to test the number of neighboring groups within a 3m radius of the focal group because subordinate *N. pulcher* preferentially visit neighboring groups within a 3m radius of their own group (Heg et al. 2008a). The density of neighboring groups was not correlated with a group’s location on the edge versus center of the colony (linear mixed model: $T_{29} = -0.61, p=0.55$), as many colonies have hard edges with dense areas that end abruptly whereas other have sparser areas that gradually thin out.

Because we also had information on extra-pair parentage for the majority of these groups (28/31 groups; see (Hellmann et al. 2015a), we used generalized linear mixed models (GzLMM) with a binomial distribution to determine 1) if a subordinate’s likelihood of reproducing within its group was predicted by its relatedness to the dominants in its group and 2) if the relatedness between the dominant male and female pair predicted the level of extra-pair parentage in the group.

Finally, to examine general dispersal patterns, we examined the relatedness of male and female subordinates to individuals within their own subpopulation (Figure 11), as well as to individuals in the other subpopulations. Because dispersal tends to be size-
biased (Stiver et al. 2007), we split subordinates into two size classes according to those suggested by Stiver et al. (2007): small reproductively mature subordinates (30-45mm SL) and large reproductively mature subordinates (45-55mm SL).

**Comparisons of RADseq and microsatellite datasets.** For 107 individuals in this dataset, we could quantify relatedness using both RADseq data (described above) and 6 highly variable microsatellites used in previous analyses of relatedness in this fish (Table 6). We assembled two datasets, each using the same 107 individuals, one with relatedness data generated from using 6 microsatellite loci and the other with the same 2,250 RADseq loci used in the full dataset. We ran the previously described relatedness analysis on both datasets (package RELATED using Wang (2002)), and compared both the reported point estimates and confidence intervals of relatedness values. All alleles in both datasets were within Hardy-Weinberg equilibrium.

**Ethical Note.** *N. pulcher* is a highly abundant cichlid species and is neither endangered nor threatened; however, we made attempts to reduce the number of fish we collected by using fish for multiple studies that addressed different questions (Hellmann et al. 2015a, Hellmann et al. 2016). New social groups had occupied the created vacated territories within a day or two post-collection. All methods, including euthanasia techniques, were approved by The Ohio State University IACUC (protocol ID 2008A0095) and the Animal Research Ethics Board of McMaster University (Animal Utilization Protocol

Results

Within-group relatedness and colony structure. Consistent with previous studies in this species (Dierkes et al. 2005, Stiver et al. 2005), we found that subordinates were more closely related to the dominant female in their group (mean ± s.e.: 0.14 ± 0.03) than to the dominant male in their group (mean 0.02 ± 0.02 s.e.; Wilcoxon rank sum: W=3535, p<0.001). Smaller subordinates were more closely related to the dominant female than were larger subordinates (CLMM: Z_{65}=3.79, p<0.001) but the body size of a subordinate did not influence its relatedness to its dominant male (CLMM: Z_{35}=0.34, p=0.73). Interestingly, subordinate males were more related to the dominant female than were subordinate females (Z_{51}=2.07, p=0.04) and tended to also be more related to dominant males than were subordinate females (Z_{35}=1.60, p=0.11).

Subordinates (both males and females) were more closely related to their dominant male breeders in groups located in the center of the colonies compared to groups along the edges of the colonies (Wilcoxon rank sum: W=823, p=0.01; Figure 12). In general we observed extremely low levels of relatedness between dominant males and subordinates for groups on the edges of the colonies: 35 of the 37 subordinates collected from edge territories were completely unrelated to the dominant male in their group (mean relatedness values ranging from -0.13 to 0.01). Relatedness between dominant females and subordinates did not vary between the center and edges of the colonies
(CLMM: $Z_{62}=0.76$, p=0.45). Relatedness between dominant females and their subordinates was negatively correlated with the density of neighboring groups ($Z_{51}=-2.08$, p=0.04; Figure 13), while relatedness between dominant males and subordinates was not related to density of neighboring groups ($Z_{35}=1.09$, p=0.35).

Within-group relatedness and extra-pair parentage. Extra-pair paternity, by subordinate males from the same group or by dominant and subordinate males from neighboring groups, was significantly less likely when the relatedness between the dominant male and female was high (GzLMM with binomial distribution: $Z_{16}=-3.20$, p=0.001). The frequency of extra-pair maternity was not correlated to the degree of relatedness between the dominant male and female ($Z_{16}=0.003$, p=0.99). Subordinate males were significantly more likely to reproduce in their group when they were more related to the dominant male (GzLMM with binomial distribution: $Z_{9}=2.16$, p=0.03) and subordinate females were significantly more likely to reproduce if they were more closely related to their dominant female ($Z_{18}=2.16$, p=0.03).

Movement between groups and colonies. RADseq allowed us to track individual movement among groups and colonies (Figure 11). Smaller female subordinates were more likely to be related to other group members than were larger female subordinates (GzLMM with binomial distribution: $Z_{14}=1.96$, p=0.05). Body size of male subordinates did not influence the probability of being related to other group members ($Z_{11}=0.58$, p=0.56).
Nearly 5% (8/168) of the individuals in our dataset had relatives in a different group within the same colony and another 5% (8/168) had kin in a group within a different colony altogether. In general, small female and male subordinates (30-45mm SL) were more related to small female and male subordinates (respectively) within their own subpopulation than to subordinates in other subpopulations (Wilcoxon rank sum; small females: W=14766, p<0.001; small males: W=12396, p=0.002). There was a similar, albeit weaker, trend for large female subordinates to also be more related to large female subordinates in their own subpopulation (45-55mm SL: W=3400, p=0.08).

However, large male subordinates were no more related to large male subordinates within their subpopulations compared to large male subordinates in other subpopulations (W=290, p=0.58).

**Comparison of RADseq and microsatellite data for estimating relatedness.** RADseq was a much more precise technique for measuring and tracking relatedness compared to microsatellite markers. While point estimates of relatedness values were correlated between the two techniques (Pearson’s correlation: \( t_{2344}=10.9, p<0.001 \)), the correlation coefficient was relatively low (Pearson’s \( r=0.22 \)).

Confidence intervals were also significantly larger with the microsatellite data than with RADseq data (Wilcoxon rank sum: \( W=479300, p<0.001 \); Figure 14). In some cases, confidence intervals were non-overlapping between the two techniques and microsatellites provided different estimates of relatedness than RADseq. Our ability to distinguish unrelated individuals from related individuals was reduced when using the
microsatellite data (Figure 14). For example, of the 53 subordinates that were present in both datasets, RADseq data classified 35 as unrelated to the dominant female, 6 as a half-sibling of the dominant female, and 12 as a full-sibling or offspring of the dominant female. Confidence intervals in all cases were small enough that classifications into these categories were not ambiguous (i.e. confidence intervals did not span multiple categories). However, using microsatellite loci, only 22 out of 53 subordinates had confidence intervals small enough that subordinates could unambiguously be assigned as unrelated, half-siblings, or full-siblings/offspring of the dominant female. For 20 subordinates, confidence intervals when using microsatellites were large enough that we were unable to distinguish between subordinates that were unrelated to the dominant female versus those that had a half-sibling relationship with the dominant female. For an additional 4 subordinates, we were unable to distinguish between subordinates that had a half-sibling relationship with the dominant female versus those that were full-siblings/offspring of the dominant female. For the remaining 7 subordinates, microsatellites classified the relationship between dominant females and subordinates differently than did the RADseq data.
Table 6: Genetic characteristics of the six loci used to run the relatedness analysis based on microsatellites. Shown are observed (H_{obs}) and expected (H_{exp}) heterozygosity, the polymorphic information contents (PIC), and the estimated frequency of null alleles for each locus. Heterozygosity, PIC, and null frequencies were calculated using CERVUS 3.0 based on genetic data from the 54 unrelated dominants from the reduced dataset.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Reference</th>
<th>Alleles</th>
<th>Range</th>
<th>H_{obs}</th>
<th>H_{exp}</th>
<th>PIC</th>
<th>Null</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOC101</td>
<td>Brandtmann et al. (1999)</td>
<td>21</td>
<td>150-195</td>
<td>0.720</td>
<td>0.875</td>
<td>0.853</td>
<td>+0.0916</td>
</tr>
<tr>
<td>TMO11</td>
<td>Zardoya et al. (1996)</td>
<td>24</td>
<td>170-230</td>
<td>0.889</td>
<td>0.885</td>
<td>0.869</td>
<td>-0.0079</td>
</tr>
<tr>
<td>TMO13</td>
<td>Zardoya et al. (1996)</td>
<td>21</td>
<td>220-280</td>
<td>0.850</td>
<td>0.855</td>
<td>0.843</td>
<td>+0.0184</td>
</tr>
<tr>
<td>TMO25</td>
<td>Zardoya et al. (1996)</td>
<td>18</td>
<td>360-415</td>
<td>0.815</td>
<td>0.832</td>
<td>0.808</td>
<td>+0.0041</td>
</tr>
<tr>
<td>UME003</td>
<td>Parker and Kornfield (1996)</td>
<td>28</td>
<td>190-265</td>
<td>0.944</td>
<td>0.913</td>
<td>0.900</td>
<td>-0.0229</td>
</tr>
<tr>
<td>US783</td>
<td>Schliewen et al. (2001)</td>
<td>25</td>
<td>160-250</td>
<td>0.852</td>
<td>0.924</td>
<td>0.910</td>
<td>+0.0327</td>
</tr>
</tbody>
</table>
Figure 11: Partial map of the 7 sampled colonies (and one additional unsampled colony), with unsampled groups as grey dots and sampled groups as black dots. After removing 3 individuals from our dataset due to low sequencing coverage, we analyzed a total of 22 dominants and 37 subordinates across 11 edge groups, and 37 dominants and 72 subordinates across 20 center groups. Lines connect kin found in different groups within the same colony, as well as different groups in different colonies. Solid lines indicate full-siblings/offspring between two groups and dashed lines indicate half-siblings between two groups. Depths of the colonies are identified on the bars lining the colony map. Numbers next to the colonies indicate groupings of the colonies into 4 smaller populations for analysis. Note that distances between the colonies on the figure are not to scale and there are additional unsampled groups that are not depicted on the map.
Figure 12: Mean relatedness (± standard error) between dominants and subordinates for groups in the center of the colony versus on the edge of the colony. Subordinates were significantly more related to the dominant male in their group when groups were located in the center of the colony compared to the edge of the colony, but relatedness between dominant females and subordinates did not vary significantly between groups on the center and edge of the colony.
Figure 13: The number of neighboring groups within a 3m radius, plotted against the relatedness values of subordinates to the dominant female within their group. As the density of neighboring groups increased, subordinates were significantly less related to dominant females within their group.
Figure 14: Using RADseq, we identified unrelated individuals from different groups (white), half-sibs from different groups (light grey), and full-sibs/offspring from different groups (dark grey). Data presented are means with 95% confidence intervals. RADseq techniques significantly improve the precision of relatedness estimates compared to microsatellites, which provided much larger confidence intervals.
Discussion

Using RADseq data, we generated over 2000 polymorphic loci, and used these to detect novel patterns of relatedness in *N. pulcher*, an emerging model species for the study of cooperation and social behavior. We found evidence that subordinate females disperse frequently, but for shorter distances and at a smaller size than did subordinate males. Further, we found that subordinates were more likely to reproduce when they were related to their same-sex dominant, corroborating restraint models of reproductive skew (Johnstone and Cant 1999). Finally, within-group relatedness varied with both the density and location of neighboring groups relative to the focal group, demonstrating that colony-level social structure can help explain variation between groups in the degree of relatedness among group members.

Relatedness and colony-level social structure. We found that both the density of neighboring groups and the location of a group on the edge versus center of the colony were connected to the degree of relatedness between dominants and subordinates in a group. Dominant males were significantly less related to subordinates within their group when their group was on the edge of the colony compared to when groups were in the center of the colony; however, relatedness between dominant females and their subordinates did not vary between territories on the edge and in the center of the colony. This colony-level variation in relatedness for dominant males, but not dominant females, is likely a result of two factors. First, rates of extra-group maternity do not vary between the edge and center of the colony, but rates of extra-group paternity are higher
on the edge of the colony (Hellmann et al. 2015a), thereby reducing the number of offspring that are descendants of the dominant male. However, this alone does not likely explain the extremely low levels of relatedness, given that dominant males on the colony edge still sire most of the offspring on their territory (Hellmann et al. 2015a). Second, it is likely that increased predation on the colony edge on dominants is male-biased. Males engage in extensive fights over territory ownership (O'Connor et al. 2015), which can make dominant males more vulnerable to predation and can reduce their tenure as a dominant. Therefore, these results suggest that males on the edge of the colony face particularly high fitness costs of living on the edge of the colony, whereas females do not appear to share these same fitness consequences of living on the colony edge.

Consistent with our predictions, we found that subordinates were less related to dominant females within their group when their group was located in a denser area of the colony. However, we did not find any effect of density on relatedness to the dominant male. These contrasting patterns may be due to differences in the ways in which males and females hold territories in this species. In this species, females hold only one territory, whereas males often hold multiple territories, particularly when territories are spatially clustered together (i.e., in denser areas; Desjardins et al. 2008a, Wong et al. 2012). Therefore, in denser areas, it is possible that subordinates may be able to more easily disperse from their natal territory and move to another territory of their father. This may be advantageous, as subordinates may face less aggression joining a group of their father compared to a group containing no kin (Brown and Brown 1993, Watson et al. 1994). In these situations, subordinates would be related to the dominant male in the group, but
would be unrelated to the dominant female in the group. Subordinate males in a closely
related species (*Neolamprologus obscurus*) adopt this strategy: they disperse from the
territory of their mother, but remain within the larger territory of their father (Tanaka et al.
2015).

**Relatedness and reproductive sharing.** We found evidence that patterns of reproductive
sharing among groups are related to variation in within-group relatedness in this species.
First, extra-pair paternity was significantly less likely when the dominant male and
female were more related. This is in contrast to our predictions and to the results of a
meta-analysis recently conducted on birds (Arct et al. 2015); however, given that no
dominant pair had relatedness beyond the level of cousins, it is likely that higher
relatedness between dominants in our dataset did not produce inbreeding depression, but
did provide benefits in terms of increasing the number of their genes passed to their
offspring (optimal outbreeding distance: Bateson 1982, Kokko 2006). It is also possible
that other non-genetic benefits of breeding with kin (e.g. increased cooperation during
parental care) outweigh any potential costs or risk associated with inbreeding depression
(Thünken et al. 2007, Stiver et al. 2008).

Both subordinate males and subordinate females were more likely to reproduce
when they were more related to the same-sex dominant. These results are in contrast to
concession and tug-of-war models of reproductive skew (Vehrencamp 1983b, Keller and
Reeve 1994, Hamilton 2013), but corroborate restraint models of reproductive skew
(Johnstone and Cant 1999). In restraint models, the degree of reproductive skew reflects
the credibility of dominant threats of eviction. Because dominants face higher costs of evicting related subordinates, restraint models predict that related subordinates are better able to successfully reproduce within their group without triggering eviction (Johnstone and Cant 1999). Further, given the frequent level of extra-group parentage in this species (Hellmann et al. 2015a), it may be impossible for dominants to completely prevent extra-pair fertilizations due to the high number of reproductive competitors within their own group and in nearby groups. In these cases, it would benefit the dominant to allow kin to reproduce rather than unrelated group members or neighbors, because dominants gain indirect fitness benefits if the offspring of their kin survive and mate (Reeve 1998).

**Dispersal patterns inferred from relatedness.** Despite a widely held notion that subordinate females rarely disperse in these fish, we found that subordinate females were less related to dominants within their group than were subordinate males. Further, we found that smaller subordinate females were more likely to be related to current group members than larger female subordinates, whereas small and large subordinate males were similarly related to current group members. Combined, these results suggest that subordinate females disperse frequently and at smaller sizes relative to subordinate males.

Given that female subordinates are more related to subordinates within their own subpopulation, it is likely that female subordinates generally disperse to nearby groups within their subpopulation, while male subordinates disperse between subpopulations. This is consistent with previous studies in this species have found evidence for male-biased dispersal between subpopulations, but no evidence for sex-biased dispersal within
a subpopulation (Stiver et al. 2007). Anecdotally, in our dataset, females accounted for nearly all recorded incidences of movement between groups within a colony, whereas males conducted nearly all recorded incidences of movement between colonies.

We suggest three potential explanations for why subordinate males and females may disperse at different sizes. First, because subordinate females disperse a shorter distance within the colony, it may be easier for them to disperse at a smaller size compared to subordinate males, who often disperse between colonies and must cross large areas of open sand without shelter where they are highly vulnerable to predators (Stiver et al. 2007). Second, it is possible that male and female subordinates disperse at the same age but because males grow more quickly than females (A. Jungwirth, pers. comm.), male subordinates are larger in body size at the time of dispersal. Finally, it may not be advantageous for small subordinate females to remain in their natal group if there are several larger subordinate females in their group. Because dominant female tenure tends to be relatively long (Dierkes et al. 2005, Stiver et al. 2007) and dominance is strictly size-based, only the largest subordinate female can inherit the territory and any smaller females in the group must wait until she dies before they can become dominant. Therefore, smaller female subordinates may benefit from establishing themselves in a nearby group with fewer female subordinates, where they have a greater chance of inheriting the dominant position. This may explain why subordinate female dispersal appears to be common despite female inheritance of territories (Stiver et al. 2006): the females with the best chance of inheriting their natal territory (large females) do not disperse, whereas females with a low likelihood of inheriting their natal territory disperse
to groups where they are more likely to inherit a breeding position. However, because subordinate males rarely inherit territories (Stiver et al. 2006), they may wait to disperse from their father’s territory until they reach a size at which they may be able to challenge for a dominant breeding position in another group or can opportunistically disperse to a vacant territory (Jungwirth et al. 2015b, Tanaka et al. 2015). Subordinates in the closely related *N. obscurus* appear to adopt similar strategies—subordinate females disperse away from their father’s territory at a smaller size, but subordinate males remain in their father’s territory, likely because subordinate males are more tolerated in the territory of their father than a territory of an unrelated male (Tanaka et al. 2015).

**Conclusions.** This study is one of the first to use RADseq data to assess within-population relatedness in social species in the wild. Relative to microsatellites, these genomic-scale data provided significantly more precise measurements of relatedness and thus show great potential for studies that estimate relatedness using genetic data in behavioral ecology. Using the thousands of loci generated with this genomic technique, we were able to uncover novel patterns of relatedness in *N. pulcher*. Specifically, we found that differences in the degree of relatedness among group members can help explain differences in the level of reproductive sharing between dominant and subordinate group members. Further, we demonstrate that the variation in the density and relative location of neighboring groups is linked to patterns of relatedness within a group. These data are in agreement with a growing literature demonstrating that the broader social context is an important determinant of social and reproductive dynamics within a
group (Bergmüller et al. 2005b, Bergmüller and Taborsky 2005, Radford 2011, Gavrilets and Fortunato 2014, Hellmann and Hamilton 2014, Vrublevska et al. 2014, Hellmann et al. 2015a, Hellmann et al. 2015b, Jungwirth and Taborsky 2015, Jungwirth et al. 2015b). Specifically, neighboring groups can influence both direct and indirect fitness gains within an individual’s own group (Hellmann et al. 2015a, Jungwirth and Taborsky 2015) as well as an individual’s ability to negotiate additional current and future fitness opportunities (Buston and Zink 2009, Shen and Reeve 2010). Therefore, further research exploring how within and between dynamics interact would improve our understanding of individual decision-making and reproductive success in group-living species.
**Chapter 6: Group Response to Social Perturbation: Impacts of Isotocin and the Social Landscape**

**Abstract:** 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 neighborhoods have greater opportunities to disperse and join a nearby group compared to individuals in sparse social neighborhoods with few nearby groups. To explore the influence of the social neighborhood 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 neighboring 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 behavior by injecting the removed individuals with isotocin, a nonapeptide hormone that has been shown in the laboratory to increase the expression of submissive behavior 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 neighborhood in determining within-group dynamics but surprisingly contradict the notion that submissive behavior reduces aggression and facilitates group stability.
Introduction

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 et al. 2002). Therefore, in group-living species there should be strong selection for behaviors that mitigate conflict and reduce disputes within the group (Aureli et al. 2002, Cant and Johnstone 2009, Bourke 2011, Thompson et al. 2014). Subordinate individuals often make use of submissive displays to appease dominant group members and increase their likelihood of being tolerated within the group (Wilson 1975, Huntingford and Turner 1987, Bergmüller and Taborsky 2005). 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 signaler to concede a conflicted resource without the need for further costly aggression (Lorenz 1966, Matsumura and Hayden 2006). They also allow the signaler to remain in the same spatial location rather than fleeing from the dominant (Issa and Edwards 2006, Matsumura and Hayden 2006, Ligon 2014). Consequently, the expression of submissive behavior is essential for the formation and maintenance of stable groups (Schenkel 1967, Hick et al. 2014).

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 et al. 2008, Kutsukake and
Clutton-Brock 2008). For example, individual subordinate members may be more expendable in larger social groups than in smaller social groups (biological market theory; Noë and Hammerstein 1994, Kutsukake and Clutton-Brock 2008). Similarly, groups living in densely populated areas may have a greater ability to attract new members from neighboring groups, which may decrease the importance of current members and consequently, decrease dominant tolerance of subordinate behavior (Noë and 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 neighboring groups can facilitate successful dispersal to another group (Bergmüller et al. 2005a, Heg et al. 2008a, Drewe et al. 2009) 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 et al. 2005a, Zöttl et al. 2013a).

In this study, we sought to understand how the external social environment, the expression of submissive behavior 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 behavior 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 et al. 2005, Stiver et
Each social group consists of a dominant breeding pair and 1–20 subordinates that jointly defend permanent territories (Wong and Balshine 2011a). Subordinates form size-based dominance hierarchies and care for the offspring of the dominant breeders (Taborsky and Limberger 1981) until reaching breeding status by inheriting their current territory (Balshine-Earn et al. 1998, Dierkes et al. 2005, Wong and Balshine 2011a) or dispersing to fill vacant breeding positions in other territories (Bergmüller et al. 2005a, 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. 2008a) and receive little aggression when visiting these nearby groups (Bergmüller et al. 2005a). Subordinate removal treatments in *N. pulcher* simulate a dereliction of cooperative duties (Wong and Balshine 2011a). Such removals also induce rank conflict among the remaining group members as they jockey for position in the perturbed hierarchy (Wong and Balshine 2011b). Removals can result in punishment from the other group members, including eviction from the group (Balshine-Earn et al. 1998, Fischer et al. 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* (Taborsky 1985, Bergmüller and Taborsky 2005, Bender et al. 2006, Bruintjes and Taborsky 2008, Reddon et al. 2011, al. 2007).
Reddon et al. 2012, Reddon et al. 2015) and appear to facilitate social stability in this species (Hick et al. 2014). To explicitly examine the role of submissive behavior 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 and Thompson 2012, Thompson and 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 behavior upon return to their group, but did not show any change in aggressive or affiliative behaviors (Reddon et al. 2012). We predicted that individuals that 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 and 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 behaviors are costly (Grantner and Taborsky 1998), we would expect subordinates to scale their use to the potential cost of eviction. Therefore, submissive behaviors should be more valuable in low-density areas where individuals have a reduced ability to disperse to neighboring groups (Bergmüller et al. 2005b, Kutsukake and Clutton-Brock 2008). We also predicted that returned subordinates would receive more aggression from dominants and suffer increased rates of eviction in denser social neighborhoods 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 (Noë and Hammerstein 1994, Cant and Johnstone 2009).

**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 behaviors produced and received. Following published ethograms for this species (Sopinka et al. 2009, Hick et al. 2014, Reddon et al. 2015), we categorized all social behaviors 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 behaviors (parallel swims, follows, soft
touches). 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 neighboring groups significantly differed between fish assigned to the removal or control treatments. Removed fish were held inside an opaque minnow bucket (6 liter) 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; following Reddon et al. (2012)). Neither group size nor the density of neighboring groups differed significantly between the control and IT-treated fish. A trained experimenter, blind to the treatment conditions, administered the injections and conducted the behavioral observations. We selected this dose of IT because it has previously been shown to increase submissive behavior in *N. pulcher* in the laboratory (Reddon et al. 2012). 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. 2015a) 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 behavioral observation in which we collected all of the same behavioral 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 behavior 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 Appendix B: Supplementary Table 3); therefore we analyzed 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 behavior (see
Appendix B: Supplementary Table 3). Therefore, for hiding behavior, we examined both control and removal treatments. For this model, we included a fixed effect of removal treatment to account for variation in behavior 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 over-dispersed. 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 (GLMM) 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 neighboring 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 behavior (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 et al. 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 et al. 2014). The effects of exogenous IT injections are short lived in *N. pulcher* (Reddon et al. 2014). All aggressive behavior observed in this study was within the range of normal behavior 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 Utilization 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 Behavior.

**Results**

**Isotocin Treatment.** Focal subordinates that received IT produced more frequent submissive displays than those that received the control saline injection treatment (Table 7, Fig. 15). Following previous work (Reddon et al. 2012, O'Connor et al. 2013, Hick et al. 2014), 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 7, Fig. 16a) and were more likely to be evicted from their groups (Table 7, Fig. 17a) 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 7, Fig. 16b). Similarly, the probability of eviction from the group increased as the density of surrounding groups increased (Table 7, Fig. 17b). 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 7). Neither group size nor the density of neighboring groups was a significant predictor of the rate of focal subordinate submission upon return to the group (Table 7).

**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 neighboring
groups increased (Fig. 18a) whereas individuals that received IT spent more time hiding as the density of neighboring groups increased (Fig. 18b).

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: $Z_{16} = -1.85, P = 0.06$).

Table 7: Best-fit models illustrating effects on aggression received from the group (GLMM), submission given by the focal subordinate (GLMM), time spent hiding post-return (linear mixed effects model) and rates of subordinate eviction/re-acceptance into the group (logistic regression). Dashes refer to factors that were not included in the best-fit models. Significant $P$ values ($\alpha<0.05$) are shown in bold.

<table>
<thead>
<tr>
<th>Predictors</th>
<th>Aggression received</th>
<th>Submission given</th>
<th>Time spent hiding</th>
<th>Eviction/re-acceptance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$Z_{13}$</td>
<td>$P$</td>
<td>$Z_{15}$</td>
<td>$P$</td>
</tr>
<tr>
<td>Pre-removal behavior</td>
<td>-2.00</td>
<td>0.046</td>
<td>-1.57</td>
<td>0.12</td>
</tr>
<tr>
<td>Isotocin treatment</td>
<td>3.69</td>
<td>&lt;0.001</td>
<td>3.55</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Density of neighbors</td>
<td>2.72</td>
<td>0.006</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Group size</td>
<td>2.01</td>
<td>0.044</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Sex</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Treatment-density</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>
Figure 15. 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).
Figure 16. 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 17.** 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).
Figure 18. 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 neighboring groups. Squares: control removal; circles: treatment removal. Both slopes differed significantly from the null hypothesis of no effect (GLME: saline: $t_{15} = -4.42, P = 0.026$; isotocin: $t_{12} = 2.64, P = 0.021$).
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 neighbors 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 neighbors 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 behavior is thought to facilitate group stability by streamlining the formation of dominance hierarchies and reducing the need for costly aggressive behavior by expeditiously settling conflicts between individuals within a social group (Wilson 1975, Drews 1993). Thus, well-developed submissive signals may be essential for the emergence and maintenance of stable social groups (Schenkel 1967, O'Connor et al. 2000, Hick et al. 2014). In this experiment, we predicted that submissive behavior from the returned subordinate would appease the remaining group members and attenuate conflict.
within the group (Bergmüller and Taborsky 2005), reducing the probability of eviction from the group. Surprisingly, we found that despite the increased submissive behavior 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.

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 (Tibbetts and Dale 2004, Dey et al. 2014) 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 signaled behaviorally 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 behavior 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 neighboring 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 (Cote and Clobert 2007, Cote et al. 2010, Aplin et al. 2014). 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 (Dingemanse et al. 2003, Krackow 2003, Cote and Clobert 2007, Harrison et al. 2014). 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 behavior (Sih et al. 2004), but we did not observe this effect.
Genetic and behavioral data showing frequent dispersal to neighboring groups (Stiver et al. 2004, Bergmüller et al. 2005a, Stiver et al. 2006, Heg et al. 2008a) strongly suggest that dispersal opportunities increase with neighbor 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 neighboring groups. Eviction threats are more credible when subordinates are easily replaced and are reinforced with aggression when subordinates fail to cooperate (Noë and Hammerstein 1994, Bergmüller et al. 2007, Kutsukake and Clutton-Brock 2008, Cant and Johnstone 2009), 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 and 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 et al. 1994; fish: Brown and Brown 1993; primates: Bernstein and 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 et al. 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 (Noë and Hammerstein 1994, Kutsukake and Clutton-Brock 2008). 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 (Schadelin et al. 2012).

We also predicted that subordinates would decrease their submissive behavior in denser areas of the colony. However, we did not find an effect of group density on submissive behavior. Alternatively, subordinates may have sought to appease dominant individuals through helping behavior (territory maintenance, broodcare and territory defense), as has been previously reported in this species (Balshine-Earn et al. 1998, Bruintjes and Taborsky 2008, Fischer et al. 2014) (Balshine et al., 1998; Bruintjes & Taborsky, 2008; Fischer et al., 2014). However, subordinates in our experiment significantly reduced rather than increased their territory defense against heterospecifics in the post-removal observation compared to the pre-removal observation. This suggests that subordinates were not using this form of helping behavior as an appeasement tactic. We could not observe broodcare and territory maintenance behaviors 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 behaviors can be observed directly would be valuable in clarifying this issue.

Individuals may use avoidance behavior rather than submission to circumvent dominant aggression in wild groups. Submission is an effective appeasement behavior 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 (Bergmüller and Taborsky 2005, Bruintjes and Taborsky 2008, Riebli et al. 2011, Hick et al. 2014). 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 and 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 (2008) 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 neighboring 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 behaviors (Macdonald and 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 and 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 behaviors (Reddon et al. 2015). Collectively, our results suggest that while IT strongly influences individual behavior and thus, group response, the influence of IT on within-group conflict and behavior 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 neighboring groups.
**Chapter 7: The Impact of Dominant Partner Choice on Helping and Eviction Dynamics in a Game Theoretic Model.**

**Abstract**

In many cooperatively breeding species, subordinates that are unrelated to dominants provide helping behavior despite receiving no indirect fitness benefits for their cooperation. Pay-to-stay mechanisms, in which subordinates provide help as a form of rent to remain on the dominant’s territory, have been shown to regulate helping dynamics in several cooperatively breeding species. In pay-to-stay, subordinates that do not help sufficiently are punished or evicted from the group by more dominant individuals. The credibility of dominant eviction threats may vary with the social context, and biological market theory principles suggest that dominants that can easily replace evicted subordinates may demand more help in exchange for continued partnership with the subordinate. Here, we develop a game theoretic model to examine how the relative cost of eviction for dominants influences the degree of help provided by subordinates and the threshold of helping behavior at which dominants are willing to evict subordinates. In this model, subordinate group members determine the level of help they will provide, while dominants decide the threshold of eviction, such that subordinates have an increasing probability of eviction if they provide help below the threshold of eviction. In agreement
with a previously published model, we found that subordinate opportunities to breed outside of the group do not influence eviction and helping dynamics under most parameter space. Similarly, dominant threats of partner choice alone are not the primary mechanism regulating market dynamics in group-living species, as increased opportunities for dominants to replace current subordinates resulted in decreased helping behavior and reduced eviction thresholds in contrast with most empirical studies. Instead, it is likely that partner choice, in tandem with other mechanisms such as negotiation or plasticity, regulates market dynamics observed in empirical systems.
**Introduction**

The evolution and stability of helping behavior in group-living species has received a great deal of attention in the last 50 years. Specifically, questions center around why subordinates help, since helping behavior is energetically costly and can reduce both survival and growth (Hamilton 1963). Kin-selected benefits of helping behavior have been suggested as the primary mechanism regulating the evolution of helping in group-living birds and mammals (Russell 2004), but in many species, subordinates that are unrelated to dominants also provide help (Cockburn 1998, Clutton-Brock 2009), suggesting that this is not a mechanism underlying subordinate helping behavior in all taxa or in all social contexts. Another suggested mechanism underlying the evolution of helping behavior is pay-to-stay (Gaston 1978, Balshine-Earn et al. 1998, Kokko et al. 2002a, Bergmüller et al. 2007), in which subordinates provide help as a means to compensate for the costs, primarily in terms of competition for reproduction and resources, that they inflict upon dominants by remaining on the territory. Pay-to-stay has been shown to regulate helping dynamics in fish (Balshine-Earn et al. 1998, Bruinjes and Taborsky 2008, Hellmann et al. 2015b) and fairy wrens (Mulder and Langmore 1993, Cockburn 1998) and has also been suggested as a potential mechanism underlying helping dynamics in other species of birds (Cockburn 1998) and meerkats (MacLeod et al. 2013).

Under the pay-to-stay theory, subordinates that do not help sufficiently are punished or evicted from the group by more dominant individuals. Theoretical models examining pay-to-stay dynamics suggest that the ability of dominants to exercise threats
of subordinate punishment and eviction has a large influence on the reproductive and social dynamics of the group (Johnstone and Cant 1999, Hamilton and Taborsky 2005b, Cant 2011). Resource holding potential, or the ability of an individual to use force within a dyadic relationship, has traditionally been used as a context-independent metric to determine the ability of dominants to credibly exercise threats (Lewis 2002); however, it is becoming increasingly clear that a dominant’s ability to punish subordinates is also context-dependent, as some resources (e.g., helping, grooming) cannot be taken by force alone (Lewis 2002). Depending on the social context, one party may have more influence than another because it has greater leverage in the social situation, even if it is weaker or socially subordinate to the other player (Cant and Johnstone 2009, Cant 2011). For example, primate females in estrus temporarily improve their position within the social network despite no formal change in dominance, because they have access to a resource (i.e. an unfertilized egg) that is highly prized by males (de Waal 1986, Lewis 2002).

The role of leverage resulting from supply and demand in partner choice is suggested to play a central role in the evolution of cooperation (biological market theory: Noë and Hammerstein 1994, 1995). Threats of leaving the interaction can exact greater cooperation from a partner than if the threatener had no other options but to stay (Noë and Hammerstein 1994, 1995, Hammerstein and Noë 2016). For example, cleaner fish are more cooperative and less likely to cheat (by eating mucus rather than scales) when clients are more easily able to switch to another cleaner (Bshary and Noë 2003). Similarly, the ability of subordinates to choose between two dominants can allow the
subordinate to negotiate for higher reproductive opportunities within its chosen group (Reeve 1998).

The same dynamics governing partner choice in the aforementioned systems likely also influence the effectiveness of eviction threats to exact helping behavior from subordinates. Eviction from a group is nearly always costly for subordinates due to elevated glucocorticoid levels (Young et al. 2006), reproductive failure (Young et al. 2006), weight loss (Young et al. 2006), reduced ability to forage (Clutton-Brock et al. 1998), and increased predation risk (Buston 2003). However, the cost of eviction for dominants may change drastically based on the social context. In many cooperatively breeding species, dominants derive fitness benefits, primarily in terms of increased offspring survival and reduced workload, from subordinate help (Dickinson and Hatchwell 2004, Russell 2004). Therefore, evicting a subordinate is expected to be costly for dominants when replacing evicted subordinates is difficult or if each subordinate makes a large contribution to group productivity (e.g. small groups). In these groups, dominants may have less leverage to exact help from subordinates because dominant costs of evicting subordinates may be similar or even greater than the cost of retaining a subordinate that does not provide sufficient help. However, in situations in which subordinates can be easily recruited from nearby groups (e.g. in dense areas) or in groups where there are already many subordinates, the loss of one subordinate does not greatly reduce group productivity. In these cases, dominants may be less tolerant of subordinate dereliction of helping behavior (Clutton-Brock et al. 2001, Hellmann et al. 2015b) and may be able to negotiate increased help from subordinates because their eviction threat is
credible (Johnstone and Bshary 2002, Bowles and Gintis 2004, Cant and Johnstone 2006). Consequently, the social context likely alters the credibility of dominance eviction threats and thus, the ability of subordinates to reduce the amount of help they provide without facing punishment.

There is empirical evidence to support the suggestion that helping behavior and eviction dynamics are influenced by the social context. Subordinates increase helping behavior when outside competition for their position in the group is higher (Bruintjes and Taborsky 2008) and when subordinates are experimentally prevented from helping, dominants evict subordinates more frequently in denser areas of the colony (Hellmann et al. 2015b). These results imply that eviction by dominants is triggered more easily when subordinates can be easily replaced and that subordinates compensate for this by increasing their helping behavior. Further, this suggests that both the amount of helping behavior and the threshold for eviction are under selection and should be responsive to the social context. Despite evidence that helping behavior is regulated by the cost of eviction for dominants (Gaston 1978, Mulder and Langmore 1993, Cant 2011), experimental evidence for this hypothesis is limited to experiments in one species of fish (N. pulcher) and one species of bird (superb fairy wrens, Malurus cyaneus). This is largely because experiments examining eviction dynamics are difficult to conduct: the most effective threats (i.e. eviction) are rarely triggered because subordinates in stable groups use both submissive and helping behavior to prevent eviction (Bergmüller et al. 2005b, Bruintjes and Taborsky 2008). Therefore, theoretical models can generate valuable predictions regarding eviction and helping dynamics that can advance our
understanding of how punishment can alter group dynamics, as well as allow us to generate predictions that can be tested empirically across a variety of taxa with different social systems.

While a past model by Hamilton and Taborsky (2005b) focused on helping and eviction dynamics relative to variation in the constraints of subordinate eviction (e.g. in the ability of subordinates to inherit territories or survive dispersal), we instead focus on helping and eviction dynamics relative to costs of eviction for dominants (i.e. the ability of dominants to replace evicted subordinates) as well as for subordinates (i.e. the ability of evicted subordinates to become dominant). Here, we develop a model to examine how the relative cost of eviction for dominants influences the degree of help provided by subordinates and the threshold of helping behavior at which dominants are willing to evict subordinates. In this model, subordinate group members determine the level of help they will provide, while dominants decide the threshold of eviction, such that subordinates have an increasing probability of eviction if they provide help below the threshold of eviction. Consequently, this model allows for conflict within groups, but with the possibility that the partnership can be terminated.

Methods

Our game theoretical model consists of two classes of players: dominant and subordinate group members who are unrelated; that is, subordinates receive no kin-selected benefits of helping and dominants suffer no indirect fitness costs of evicting a subordinate. For the purposes of this model, we assume subordinates are large enough to
become dominant. Because large subordinates likely have difficulty being accepted into a new group as a subordinate (Wong et al. 2007), we assume that eviction from a group results in either becoming a dominant in a different group or dying.

Both parties have a shared incentive to resolve conflict via the benefits of remaining in a productive group. For subordinates, the benefits of remaining in the group are two-fold: the mortality rate of living in a group ($\mu_g$) is much lower than the mortality rate of leaving the group or being evicted from the group ($\mu_e$). Additionally, the likelihood of becoming a dominant when they live in a group ($a_g$) is higher than the likelihood of becoming a dominant if evicted from the group ($a_e$) because getting evicted results in losing the opportunity to inherit their current territory. If subordinates are able to survive long enough, they obtain a dominant breeding position and gain the fitness benefits associated with dominance in a group. Therefore, the fitness of subordinates who remain in the group ($W_{SS}$):

$$W_{SS} = (1 - \mu_g)[(a_g \bar{W}_D) + (1 - a_g)(W_{SS}) - hc]$$

where $a$ is the likelihood of becoming a dominant, $h$ is the amount of help provided by the subordinate, $c$ is the cost of helping, and $\bar{W}_D$ is the average fitness of dominants in the population given population level values for $h$ and $p (\bar{h}, \bar{p})$. This rearranges to:

$$W_{SS} = \frac{(1 - \mu_g)(a_g \bar{W}_D - hc)}{a_g + \mu_g - \mu_g a_g}$$

The fitness payoffs to a subordinate who gets evicted ($W_{SE}$) is:

$$W_{SE} = (1 - \mu_e)(a_e \bar{W}_D - e_s)$$

where $e_s$ is the cost of being evicted for the subordinate (e.g. physical injury, stress) and $\bar{W}_D$ is the average fitness of dominants in the population, given population level values.
for $h$ and $p$ ($\bar{h}, \bar{p}$). This equation implicitly states that evicted subordinates who do not attain a dominant position will die. Therefore, the total fitness for a subordinate ($W_s$) is:

$$W_s = p_e W_{SE} + (1 - p_e)(W_{SS})$$

The probability function determining the likelihood of subordinate eviction is specified as $p_e = 1/2 + 1/2 \cdot \tanh ((p - h) \cdot k)$, where the probability of subordinate eviction increases as the level of helping behavior ($h$) falls below the threshold of eviction ($p$).

Using a probability function allows for the possibility that dominants do not have perfect information or may make errors by evicting subordinates that are helping sufficiently or retaining subordinates that are lazy (Hamilton and Taborsky 2005b). At the point where $h=p$, the probability of eviction is 50%. The parameter $k$ determines the steepness of the curve, such that higher values of $k$ decrease the probability of getting evicted if $h>p$. Both $h$ and $p$ range from zero to one.

![Figure 19](image.png)

**Figure 19:** The function ($p_e = 1/2 + 1/2 \cdot \tanh ((p - h) \cdot k)$) specifying the probability of eviction ($p$) given the level of helping behavior ($h$).
For dominants, subordinate membership in the group raises the productivity of the group \((G)\) by a measure proportional to the amount of help subordinates provide. Therefore, the fitness of a dominant whose subordinate stays in the group is:

\[
W_{DS} = (1 - m)((1 + h)G - s)
\]

where \(m\) is the mortality rate of the dominant and \(s\) is the cost subordinates impose on dominants. However, if the dominant evicts the subordinate, its fitness is dependent upon the probability that it is able to replace the evicted subordinate \((n)\) as well as the cost of eviction for the dominant \((e_d)\). Dominants who evict subordinates can replace them from a population of floater subordinates have never been in a group and who are queuing to join a dominant. These replacement subordinates from the floater population will provide helping behavior at the population-level value of helping \((\bar{h})\). If the dominant evicts the subordinate, we assume that if the evicted subordinate is replaced, the dominant can also evict the replaced subordinate. Therefore, the fitness of the dominant that evicts subordinates is recursive, as it is dependent upon the average dominant fitness in the population, assuming the population average level of helping behavior \(\bar{h}\):

\[
W_{DE} = n\bar{W}_D + (1 - n)(1 - m)(G - e_d)]
\]

Therefore, dominant fitness is overall:

\[
W_D = p_eW_{DE} + (1 - p_e)(W_{DS})
\]

In this model, there are two targets of selection: \(h\), the amount of help provided, is under selection for subordinates, and \(p\), the threshold of helping behavior at which subordinates get evicted, is under selection for dominants. We solve for the evolutionarily
stable values of these variables by finding the values of \( h \) and \( p \) that satisfy the following conditions:

\[
\frac{\partial W_S}{\partial h} \bigg|_{h=\tilde{h}=h^*, p=\tilde{p}=p^*} = 0 \quad \frac{\partial W_D}{\partial p} \bigg|_{h=\tilde{h}=h^*, p=\tilde{p}=p^*} = 0
\]

\[
\frac{\partial^2 W_S}{\partial h^2} \bigg|_{h=\tilde{h}=h^*, p=\tilde{p}=p^*} < 0 \quad \frac{\partial^2 W_D}{\partial p^2} \bigg|_{h=\tilde{h}=h^*, p=\tilde{p}=p^*} < 0
\]

**Results**

*Eviction dynamics and helping behavior.* Helping behavior does not vary as \( k \), or the steepness of the eviction probability function \( p_e \), increases; however, as \( k \) increases, \( p \) approaches the value of \( h \), such that when \( k \) is high and the probability curve is steep, the threshold of eviction is only slightly below the helping behavior provided by the subordinate. However, the probability of eviction (i.e., \( p_e \) evaluated at \( h^* \) and \( p^* \)) remains constant and nearly zero across all values of \( k \) (Appendix C: Supplementary Figure 1).

Both helping behavior and the threshold of eviction are highest when the probability of replacing the subordinate \( (n) \) is zero, and both decrease as \( n \) increases, until the threshold of eviction reaches zero at moderate values of \( n \) (Figure 20A). When the cost of the subordinate to the dominant \( (s) \) is low, the threshold for eviction \( (p)=0 \). While helping remains above the eviction threshold for all values of \( s \) (and the probability of eviction remains close to zero), both helping behavior and the threshold of eviction increase as \( s \) increases. The amount of help provided by the subordinate only exceeds \( s \) when \( n=0 \). For all other values of \( n \), subordinates do not provide enough help to compensate for the costs they impose on dominants (Figure 20A).
Subordinate helping and the threshold of eviction are both high when cost of helping to the subordinate \((c)=0\) and then drop as \(c\) increases. Both helping behavior and the threshold of eviction are relatively stable until the costs of help are high, at which point the threshold of eviction increases and exceeds subordinate helping behavior. This results in close to 100% probability of eviction at high values of \(c\) (Figure 20B).

As group productivity \((G)\) increases, both the threshold of eviction and subordinate helping decrease, but subordinate helping stays above the threshold of eviction and the probability of eviction remains low. The threshold of eviction decreases as the immediate cost of eviction for dominants \((e_d)\) increases, such that when \(e_d\) reaches a moderate proportion of group productivity, the threshold of eviction reaches zero (Appendix C: Supplementary Figure 2). Subordinate helping remains consistently above the threshold of eviction for all values of \(e_d\), such that eviction is unlikely with any value of \(e_d\). Helping behavior and the threshold of eviction do not vary with the value of the immediate cost of eviction for subordinates, \(e_s\), such that helping behavior always exceeds the threshold of eviction and the probability of eviction is near zero for all values of \(e_s\) (Appendix C: Supplementary Figure 2).

When group productivity \((G)\) is moderate relative to the cost of the subordinate’s presence \((s)\), helping behavior does not vary with the probability of an subordinate becoming dominant if evicted \((a_e)\) and the probability of eviction remains near zero for all values of \(a_e\) (Figure 20C). Further, when group productivity is moderate, helping behavior is lower than the threshold of eviction when the subordinate’s probability of becoming dominant if it remains in the group \((a_g)\) is low. As \(a_g\) increases, the threshold
of eviction lowers and subordinate eviction is unlikely (Figure 20C). These results are similar if \( a_g \) and \( a_e \) vary independently (such that \( a_e \) can exceed \( a_g \)) or if \( a_e \) is held at a proportion of \( a_g \) (such that \( a_e \) is always less than \( a_g \)).

When \( G \) is low relative to \( s \) (i.e., \( s \) is a high proportion of total group productivity), subordinates are evicted at low values of \( a_g \). At higher values of \( n \), the threshold of eviction falls below helping behavior at increasingly lower values of \( a_g \) (Figure 21A). If \( a_e \) varies as a proportion of \( a_g \), such that \( a_e \) is always lower than \( a_g \), then subordinates are also evicted at low values of \( a_e \) (patterns identical to those depicted in Figure 21A). However, when \( a_e \) and \( a_g \) vary independently, such that \( a_g \) can exceed \( a_e \), the threshold of eviction is below subordinate helping at low values of \( a_e \) and exceeds helping behavior as \( a_e \) increases. The point at which \( p > h \) is dependent upon the probability that the evicted subordinate is replaced (\( n \)): as \( n \) increases, subordinates are evicted at higher values of \( a_e \) (Figure 21B). As a consequence, when \( s \) is a low to moderate proportion of \( G \), groups are likely to disband only when \( a_g \) is very low; however, when \( s \) is a high proportion of \( G \), groups are likely to disband over a wider range of \( a_g \) and also when \( a_e \) is high relative to \( a_g \).

Helping behavior and the threshold of eviction do not vary with the mortality rate of evicted subordinates (\( \mu_e \)) and the probability of eviction is close to zero for all values of \( \mu_e \). Helping behavior and the threshold of eviction remain steady until the mortality rate of subordinates within the group (\( \mu_g \)) is high. At this point, the threshold of eviction increases sharply and helping behavior falls below the threshold of eviction, such subordinates will be evicted with close to a probability of 1 (Figure 20D). Both
subordinate helping and the threshold of eviction increase sharply as dominant mortality increases; at moderate levels of dominant mortality, $p=1$ and subordinate eviction is highly likely.
Figure 20: The level of subordinate helping (solid line) and threshold of eviction (dashed line) as they vary with: A) the probability of dominants replacing evicted subordinates \((n)\) when the cost of subordinates to dominants \((s)\) is 20%, 40% and 60% of group productivity \((G)\), B) the cost of helping \((c)\), C) the probability of a subordinate becoming dominant if in a group (black) or if evicted (grey), and D) subordinate mortality within the group \((\mu_g)\). For all panels, parameters that are not varied are \(G=1, c=0.1, m=0.1, s=0.4, k=50, e_d=0.05, e_s=0.05, \mu_g=0.2, \mu_e=0.4, n=0.1, a_g=0.5, a_e=0.25\).
Figure 21: Values of helping behavior and eviction threshold across all values of $a_g$ (A) and $a_e$ (B) when group productivity is low and when the probability of replacing the subordinate ($n$) is 20%, 40% and 60%. When helping behavior is much lower than the eviction threshold, the group is likely to disband. For all panels, parameters that are not varied are $G=0.5, c=0.1, m=0.1, s=0.4, k=50, e_d=0.05, e_e=0.05, \mu_g=0.2, \mu_e=0.4,$ and $n=0.1$. $a_g$ and $a_e$ vary independently of each other.
Discussion

In this model, we assume that subordinates control the amount of help that they provide within a group, and dominants specify a threshold of eviction, such that groups will disband when subordinate helping behavior is far below the threshold of eviction and stay together when helping is much higher than the eviction threshold. Here, our model demonstrates that the relative costs of eviction for dominants alters both helping and eviction dynamics. We found that as the probability of replacing an evicted subordinate \((n)\) increases, both the threshold of eviction and subordinate helping behavior decrease and the probability of eviction remains near zero across all values of \(n\). This decrease in helping behavior and eviction threshold with increasing \(n\) is in contrast to the predictions of biological market theory, which posits that as the options for partner choice increase (or as the cost of switching partners or ending a partnership decreases), individuals should be less tolerant of their current partner and can demand more help or cooperation in exchange for a continued partnership (Noë and Hammerstein 1994, Bshary and Noë 2003, Hammerstein and Noë 2016).

Partner choice is an undeniably important mechanism regulating cooperative dynamics in interspecies mutualistic interactions, including cleaner fish-client relationships (Bshary and Noë 2003), acacia tree defense by ants (Janzen 1966), and rhizobia-plant symbiosis (Kiers et al. 2003). Mounting empirical evidence also suggests that market dynamics play a critical role in determining cooperation among group members, regulating grooming dynamics in primates (Barrett et al. 1999, Barrett et al. 2002) and intrasexual conflict in meerkats (Kutsukake and Clutton-Brock 2008). Further,
results from one experimental study in the cooperatively breeding cichlid, *Neolamprologus pulcher*, support the idea that helping dynamics are regulated by the partner choice offered by neighboring groups. Hellmann et al. (2015b) removed subordinates from their group to simulate a dereliction of helping behavior and found that subordinates from groups in denser areas of the colony were evicted more frequently, demonstrating that dominant threshold for eviction is lower when there are many neighboring groups (and presumably, their ability to replace evicted subordinates is high).

Given this strong empirical evidence for biological market theory, why did our model find that increased opportunities to replace current partners were associated with decreased help and eviction threshold? We suggest that there may not be benefits of partner choice in all social situations. In this model, the continual effort of subordinates to join the dominant imposes high costs on the dominant, because subordinates rarely provide enough help to compensate for the costs they impose on dominants. However, the immediate costs associated with eviction means that evicting uncooperative subordinates is unlikely to benefit dominants, particularly when the probability of replacing subordinates is high. In our model, there is no variation in helping ability of subordinates at equilibrium, such that evicted subordinates are replaced with subordinates that demonstrate the same level of helping behavior. This means that dominants that are favored to evict one subordinate will continually evict replacement subordinates and consequently, suffer multiple costs of evicting subordinates. Therefore, there is little incentive for dominants to invest in evicting subordinates when the probability of replacing the subordinate is high, even though current subordinates are costly.
Our assumption that all subordinates are identical and provide the same level of helping behavior is artificial, as we know that there are persistent differences among subordinates in their tendency to provide help, either due to intrinsic qualities such as personality or due to state-dependent factors such as phenotypic plasticity (Bergmüller et al. 2010). However, even if there are differences in cooperative tendencies among subordinates, the trend we observed in our model is expected to persist unless there is a high probability that a replacement subordinate will be more cooperative than the current subordinate. Just as it is costly for females to choose among many potential mates (Vitousek et al. 2007), it may be costly for dominants to choose among potential subordinates. Therefore, the costs associated with choice may rise as the number of options increases, particularly if those options only vary slightly in phenotype and cooperative tendency. Nevertheless, future models incorporating heterogeneity in partner phenotype because of consistent differences between individuals or plastic responses to partners (e.g., Hamilton 2013) would elucidate the extent to which differences in helping behavior account for market dynamics observed in empirical studies.

Similar to Hamilton and Taborsky (2005b), we found that, under most parameter space, helping behavior is not enforced by eviction threats unless subordinates impose costs on dominants. Further, unrelated helpers rarely compensate for the cost that they impose on dominants. In this model, we did not specify what that cost was to dominants, but in many cooperatively breeding species, subordinates can reduce dominant fitness by gaining a share of reproduction within the group (Clutton-Brock et al. 2001, Hellmann et al. 2015a) or by forcing dominants to invest energy and time into suppressing subordinate
reproduction (Clutton-Brock et al. 2010). Further, subordinates may compete for food with dominant group members, which may reduce the ability of dominants to reproduce (Wittig and Boesch 2003). While the level of help rose with increasing costs of the subordinate to the dominant, subordinate help (and therefore, the benefit that it conferred to dominants) was only higher than subordinate costs to dominants when the probability of replacing the subordinate was zero or when there was no cost of helping to the subordinate. Therefore, although subordinates partially compensate for imposing higher costs on dominants by increasing helping behavior, this still results in net negative fitness for dominants in most contexts. Consequently, as Hamilton and Taborsky (2005b) suggest, mechanisms aside from the threat of eviction, such as relatedness, may explain the net fitness benefits of subordinate help in many species.

Also in line with the results of Hamilton and Taborsky (2005b), we found that the mortality rate of evicted subordinates does not influence helping and eviction dynamics. We found that an evicted subordinate’s ability to breed outside of the group \((a_e)\) influenced helping and eviction dynamics, but only when the cost of the subordinate’s presence \((s)\) is a high proportion of group productivity \((G)\). When group productivity is moderate to high, subordinate eviction was unlikely at all values of \(a_e\); however, when group productivity is low relative to \(s\), the group disbands when \(a_e\) exceeds \(a_g\), and therefore, the options to breed once evicted exceed the options to breed if they remain in the group. This is not a biologically realistic scenario for most cooperatively breeding species, as subordinates are expected to voluntarily leave their group (and therefore, avoid costs associated with eviction) if there are higher opportunities to breed elsewhere.
However, this does suggest that subordinate outside options may be important in terms of subordinate threats to voluntarily leave the group and dominant incentives to retain current subordinates. Consequently, future models exploring subordinate helping behavior when subordinates can voluntarily leave the group, rather than be evicted, would help better elucidate the conditions under which subordinate opportunities to breed outside of the group regulate helping dynamics.

Game theory models examining market dynamics can be useful for clarifying trends observed in empirical studies and isolating factors that are often correlated in wild systems. For instance, Hellmann et al. (2015b) found that increased density of neighboring groups (and presumably, higher partner choice) was associated with high eviction thresholds; however, this trend could be due to variation in relatedness (Chapter 5) or personality (Cote and Clobert 2007, Cote et al. 2010, Aplin et al. 2014) due to social structure, as well as due to market dynamics.

Here, we have demonstrated that dominant partner choice alone is not the primary mechanism regulating market dynamics in group-living species, as increased opportunities for dominants to replace current subordinates resulted in decreased helping behavior and reduced eviction thresholds in our model. Consequently, future game theoretic models examining market dynamics should explore partner choice in tandem with other dynamics, such as negotiation or plasticity. In reproductive skew models, subordinates who initiate bidding games between dominants can achieve higher levels of reproduction (Reeve 1998) and this type of negotiation game, as opposed to a single sealed bid game, may lead to different dynamics in reference to helping behavior and
eviction. Similarly, models incorporating plasticity allow adjustments to a partner’s optimal strategy and can provide different solutions than traditional games where partners lack information about each other’s strategy (Taylor et al. 2006, Hamilton 2013). Therefore, integrating a wide range of modeling strategies can help clarify dynamics regulating social and reproductive dynamics in cooperatively breeding species. Collectively, the results of this model demonstrate that when the costs of choice increase with opportunities for choice, cooperative dynamics can diverge strongly from patterns that we would expect when there are benefits to increased choice. Consequently, integrating the dynamics associated with costs of increased choice with variation in partner attributes is expected to improve our understanding of market dynamics in cooperatively breeding systems.
Chapter 8: Conclusion

Here, I demonstrate the importance of considering the broader social context when exploring individual decision-making and group-level social and reproductive dynamics. Specifically, I use laboratory groups of *N. pulcher* to demonstrate that subordinates are more aggressive toward predators when groups have neighbors compared to when groups are isolated (Chapter 2) and that intragroup network structure is different when neighbors are present compared to when they are absent (Chapter 3). I also found that both reproductive sharing and relatedness in wild groups of *N. pulcher* are influenced by the density of neighboring groups as well as the relative location of a group on the edge versus center of the colony (Chapters 4 and 5). Further, I found that group composition seems to be less stable when opportunities for intergroup movement are greater: dominant threats of eviction are triggered more easily in denser areas of the colony (Chapter 6). However, my game theoretic model actually predicted that increased dominant ability to replace evicted subordinates will result in decreased subordinate help and lower eviction thresholds (Chapter 7), suggesting the dominant partner choice alone does not account for the patterns observed in Chapter 6.

In laboratory groups of *N. pulcher*, I found evidence that conflict among group members is altered by the presence of neighbors (Chapters 2-3). Specifically, conflict was higher among similarly-sized group members when neighbors were present compared to
when neighbors were absent (Chapter 3), suggesting that the presence of neighbors may destabilize the dominance hierarchy and induce rank-related conflict among group members that occupy adjacent social ranks (Hamilton et al. 2005, Wong et al. 2007). Further, I found that a larger proportion of dominant aggression was directed toward subordinates when neighbors were present (Chapter 3), suggesting that dominants are less tolerant of subordinates within their group in the presence of neighbors. Supporting this, I found that subordinates, in addition to receiving a greater proportion of within-group aggression when neighbors were present, also provided more help (i.e. more defense) when neighbors were present (Chapter 2). Because neighboring groups provide a pool of subordinates that can potentially join the group, neighbors provide opportunities for dominants to replace current subordinates. This may allow dominants to demand more help from current subordinates in exchange for their continued presence on the territory (Noë and Hammerstein 1994, Hammerstein and Noë 2016).

In addition to evidence that conflict between dominants and subordinates is greater in the presence of neighboring groups, I also found evidence that conflict among group members is higher in denser areas of wild colonies. Subordinate eviction threats are triggered more easily in denser areas of the colony, implying that dominants are less tolerant of subordinate dereliction of helping behavior when there are many neighboring groups (Chapter 6). Further, subordinate males were more likely to reproduce in denser areas of the colony, suggesting that reproductive conflict among group members may be greater in high-density areas (Chapter 4). Finally, groups in denser areas of the colony had lower levels of relatedness between dominant females and subordinates (Chapter 5);
as conflict tends to be greater in groups of unrelated individuals (Johnstone and Cant 1999, Kutsukake and Clutton-Brock 2008), groups in denser areas of the colony may be less stable.

I also found that dominant males on the edge of the colony gain less direct and indirect fitness within their group than dominant males in the center of the colony. Dominant males on the edge of the colony sired fewer offspring within their group than males in the center of the colony (Chapter 4). Further, dominant males were significantly less related to subordinates within their group when their group was located on the edge of the colony compared to the center (Chapter 5). It is possible that males on the edges of colonies gain greater reproductive fitness in neighboring groups and therefore, have relatively similar reproductive fitness compared to males in the center of the colony. Therefore, future studies examining dominant fitness gains within their own groups as well as in other groups would help elucidate relative fitness gains of dominants on the edge versus center of the colony.

While kin-selected benefits of helping have been suggested as a mechanism regulating the frequency of helping behavior in both birds (Dickinson and Hatchwell 2004) and mammals (Russell 2004), my results suggest that hidden threats of eviction from the group are also important regulators of helping dynamics, as suggested by Cant and Johnstone (2009) and Hamilton and Taborsky (2005b). When subordinates were experimentally removed to simulate a dereliction of helping behavior, dominant threats of eviction were triggered more easily in denser areas of the colony, where evicted subordinates are possibly replaced more easily (Chapter 6). However, results of my

153
game theoretical model demonstrate that dominant ability to replace evicted subordinates should actually result in decreased help and lower eviction thresholds (Chapter 7), the opposite of what I observed in Chapter 6. Therefore, it is possible that other patterns are correlated with a high density of neighboring groups, such as more aggressive individuals (Cote and Clobert 2007, Cote et al. 2010, Aplin et al. 2014) or reduced relatedness among group members (Chapter 6), and may account for the trends observed in the wild. Further, it is also possible that the model did not capture important dynamics in wild systems that account for the patterns observed. For example, subordinates may vary in their cooperative phenotype, such that dominants can replace evicted subordinates with more cooperative subordinates. Additionally, subordinates in natural systems may negotiate helping behavior with dominants or respond plasticly to dominant strategies, such that subordinates and dominants can adjust to each other’s optimal strategy. Consequently, further models integrating these dynamics with partner choice would be useful in elucidating the mechanisms underlying empirical observations.

Collectively, this research provides strong support that within-group social and reproductive dynamics are altered by the broader social context. We have long known that social interactions within a group are regulated by the broader ecological environment—social interactions within groups are correlated with ecological variables such as rainfall (Godfrey et al. 2013), predator frequency (Edenbrow et al. 2011), and food distribution (Tanner and Jackson 2012), which can influence the stability of social relationships and the frequency of social interactions. Further, theoretical models within the last 30 years have predicted that the broader ecological and social environments are
important regulators of within-group dynamics by influencing the ability of individuals to move between groups and achieve independent breeding opportunities (Vehrencamp 1983b, Vehrencamp 1983a, Buston and Zink 2009, Cant and Johnstone 2009). However, it is only recently that empirical studies have begun to test the predictions of these theoretical models.

Within the last few years, a small number of empirical studies have demonstrated the importance of considering the influence of neighboring groups when evaluating within-group social and reproductive dynamics. In both birds and fish, the presence of out-group threats promotes social cohesion among group members (Radford 2008, 2011, Bruintjes et al. 2016). Further, previous studies in *N. pulcher* have demonstrated that perceived demand for subordinates alters social dynamics between dominants and subordinates and that group productivity is mediated by the density of neighboring groups (Jungwirth and Taborsky 2015). Therefore, the results of this dissertation add to a small, but burgeoning literature demonstrating that within-group dynamics are dependent on the presence, density, and location of neighboring groups.

Importantly, I also demonstrate that the influence of neighboring groups is not consistent across all social contexts. Rather, individual characteristics such as sex and status, as well as group characteristics such as group size, determine the extent to which group members alter their behavior in response to the presence of neighboring groups. For instance, subordinates, but not dominants, alter their defense against the predator when neighbors were present (Chapter 2). Similarly, male reproductive patterns were much more strongly influenced by the density and location of neighboring groups than
female reproductive patterns (Chapter 4). This demonstrates that social systems are highly complex, with individuals adjusting their behavior based on their own attributes, the behavior of other group members, and the social dynamics between groups. Therefore, understanding variation in behavioral and reproductive dynamics among groups and between populations necessitates further exploration as to how individual, group, and colony-level social structure interact to influence individual fitness.
References


Christensen, R. H. B. 2012. ordinal—regression models for ordinal data.


Ligon, R. A. 2014. Defeated chameleons darken dynamically during dyadic disputes to decrease danger from dominants.


Appendix A: Supplementary Tables, Chapter 4

Table 8. Genetic characteristics of the five loci used to assign parentage. Shown are observed ($H_{\text{obs}}$) and expected ($H_{\text{exp}}$) heterozygosities, the polymorphic information contents (PIC), non-exclusion probabilities (ability to identify the real parent or parental pair out of the population given allele frequency and diversity), and the estimated frequency of null alleles for each locus. Values were calculated using CERVUS 3.0 with a sample of 74 unrelated individuals (all parents) from the population.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Alleles</th>
<th>Range</th>
<th>$H_{\text{obs}}$</th>
<th>$H_{\text{exp}}$</th>
<th>PIC</th>
<th>Non-excl$_{1P}$</th>
<th>Non-excl$_{PP}$</th>
<th>Null</th>
</tr>
</thead>
<tbody>
<tr>
<td>TmoM11</td>
<td>27</td>
<td>170-230</td>
<td>0.878</td>
<td>0.891</td>
<td>0.878</td>
<td>0.645</td>
<td>0.980</td>
<td>+0.0030</td>
</tr>
<tr>
<td>TmoM13</td>
<td>24</td>
<td>220-280</td>
<td>0.865</td>
<td>0.937</td>
<td>0.926</td>
<td>0.859</td>
<td>0.991</td>
<td>+0.0366</td>
</tr>
<tr>
<td>TmoM25</td>
<td>21</td>
<td>360-415</td>
<td>0.838</td>
<td>0.818</td>
<td>0.795</td>
<td>0.482</td>
<td>0.947</td>
<td>-0.0245</td>
</tr>
<tr>
<td>UME003</td>
<td>28</td>
<td>190-165</td>
<td>0.905</td>
<td>0.917</td>
<td>0.905</td>
<td>0.706</td>
<td>0.987</td>
<td>+0.0033</td>
</tr>
<tr>
<td>US783</td>
<td>27</td>
<td>160-250</td>
<td>0.838</td>
<td>0.936</td>
<td>0.925</td>
<td>0.752</td>
<td>0.991</td>
<td>+0.0502</td>
</tr>
</tbody>
</table>

Table 9. Means and standard errors of the measured within-group characteristics (territory size [m$^2$], group size, and the number of reproductively mature male and female subordinates per group) and measured individual characteristics of the dominants within the group (dominant male and female standard length [mm] and the relative difference in size between the dominants ([dominant male SL-dominant female SL]/dominant male SL). None of these characteristics differed significantly between the edge and the center of the colony or with the density of neighboring groups.

<table>
<thead>
<tr>
<th>Predictors</th>
<th>Location Predictors</th>
<th>Location Density Predictors</th>
<th>Density Predictors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Individual Characteristics</td>
<td>Mean (s.e)</td>
<td>Test stat.$_{34}$ p-value</td>
<td>Test stat.$_{34}$  p-value</td>
</tr>
<tr>
<td>Dominant male SL</td>
<td>60.6 (0.34)</td>
<td>0.84</td>
<td>0.36</td>
</tr>
<tr>
<td>Dominant female SL</td>
<td>53.6 (0.39)</td>
<td>-0.98</td>
<td>0.34</td>
</tr>
<tr>
<td>Dominant size difference</td>
<td>0.11 (0.008)</td>
<td>1.11</td>
<td>0.28</td>
</tr>
<tr>
<td>Group Characteristics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Territory size</td>
<td>0.22 (0.02)</td>
<td>0.32</td>
<td>0.75</td>
</tr>
<tr>
<td>Group size</td>
<td>9.31 (0.50)</td>
<td>0.41</td>
<td>0.68</td>
</tr>
<tr>
<td>Male subordinates</td>
<td>1.25 (0.24)</td>
<td>-0.02</td>
<td>0.98</td>
</tr>
<tr>
<td>Female subordinates</td>
<td>1.47 (0.20)</td>
<td>-1.44</td>
<td>0.16</td>
</tr>
</tbody>
</table>
Table 10. Test statistics (with df) and p-values from full models measuring the effect estimates of location (centre versus edge), density of neighbouring groups, group size, relative size of dominant male to dominant female ([dominant male SL-dominant female SL]/dominant male SL), and the number of male subordinates on the number of offspring assigned to dominant and subordinate males within the sampled group (GLMER) and number of fathers contributing to the brood (CLM). These are data from Table 2 excluding two groups in which the dominant male gained no paternity. All significant results were robust to these exclusions.

<table>
<thead>
<tr>
<th>Predictors</th>
<th>Dom. male reproduction</th>
<th>Sub. male reproduction</th>
<th>Number of fathers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Test stat.</td>
<td>p-value</td>
<td>Test stat.</td>
</tr>
<tr>
<td>Location</td>
<td>3.04</td>
<td>0.002*</td>
<td>-2.81</td>
</tr>
<tr>
<td>Density</td>
<td>-1.03</td>
<td>0.30</td>
<td>2.57</td>
</tr>
<tr>
<td>Group size</td>
<td>1.28</td>
<td>0.20</td>
<td>-1.47</td>
</tr>
<tr>
<td>Size difference</td>
<td>1.98</td>
<td>0.05*</td>
<td>-1.74</td>
</tr>
<tr>
<td>Male subs</td>
<td>-0.63</td>
<td>0.53</td>
<td>3.25</td>
</tr>
<tr>
<td>Offspring</td>
<td>8.58</td>
<td>&lt; 0.001*</td>
<td>-1.98</td>
</tr>
<tr>
<td>Location * group size</td>
<td>-3.51</td>
<td>&lt; 0.001*</td>
<td>3.04</td>
</tr>
</tbody>
</table>

Table 11. Test statistics (with df) and p-values from full models measuring the effect estimates of location (centre versus edge), density of neighbouring groups, group size, relative size of dominant male to dominant female ([dominant male SL-dominant female SL]/dominant male SL), and the number of female subordinates on the number of offspring assigned to dominant and subordinate females within the sampled group (GLMER) and the total number of mothers contributing to the brood (CLM). These are data from Table 1 excluding the two groups in which the dominant female gained no maternity. All significant results were robust to these exclusions, except for the observed effect of the relative size difference between the dominant male and female on the number of offspring assigned to dominant female.

<table>
<thead>
<tr>
<th>Predictors</th>
<th>Dom. female reproduction</th>
<th>Sub. female reproduction</th>
<th>Number of mothers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Test stat.</td>
<td>p-value</td>
<td>Test stat.</td>
</tr>
<tr>
<td>Location</td>
<td>1.12</td>
<td>0.26</td>
<td>-2.84</td>
</tr>
<tr>
<td>Density</td>
<td>0.57</td>
<td>0.57</td>
<td>0.90</td>
</tr>
<tr>
<td>Group size</td>
<td>0.86</td>
<td>0.39</td>
<td>1.26</td>
</tr>
<tr>
<td>Size difference</td>
<td>1.32</td>
<td>0.19</td>
<td>-1.14</td>
</tr>
<tr>
<td>Female subs</td>
<td>1.21</td>
<td>0.23</td>
<td>-2.42</td>
</tr>
<tr>
<td>Offspring</td>
<td>4.56</td>
<td>&lt; 0.001*</td>
<td>3.71</td>
</tr>
<tr>
<td>Location * female subs</td>
<td>-2.71</td>
<td>0.007*</td>
<td>3.35</td>
</tr>
</tbody>
</table>
Appendix B: Supplementary Table, Chapter 6

In the control treatment (removal of 5 min), there was no change in the amount of aggression that the subordinate received from its social group, or in the amount of submission or aggression given by the focal subordinate. However, the amount of time that the focal fish spent hiding was higher after being returned to the group, which is probably a result of stress induced by the capture and handling procedure. In the removal treatment (removal of ~4 h), saline-treated individuals showed no change in the amount of aggression that they received from their group or in the amount of submission given by the focal subordinate. In contrast, isotocin (IT)-treated individuals received significantly more aggression and showed significantly more submission after being returned to their group. Subordinates in the removal treatment also showed significantly less heterospecific aggression after being returned compared to the pre-removal observations, irrespective of their hormone treatment. As with the removal controls, focal fish in the removal treatment spent more time hiding after their return to the group, regardless of hormone treatment. Significant $P$ values are shown in bold.
Table 12. Comparison between pre- and post-removal behaviour (Mann–Whitney $U$ tests) for all experimental treatment combinations

<table>
<thead>
<tr>
<th>Predictors</th>
<th>Control/Saline</th>
<th>Control/Isotocin</th>
<th>Removal/Saline</th>
<th>Removal/Isotocin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$U$</td>
<td>$P$</td>
<td>$U$</td>
<td>$P$</td>
</tr>
<tr>
<td>Aggression received</td>
<td>61</td>
<td>0.38</td>
<td>62</td>
<td>0.36</td>
</tr>
<tr>
<td>Submission given</td>
<td>68.5</td>
<td>0.14</td>
<td>55.5</td>
<td>0.70</td>
</tr>
<tr>
<td>Time spent hiding</td>
<td>17.5</td>
<td>0.01</td>
<td>21</td>
<td>0.02</td>
</tr>
<tr>
<td>Aggression given</td>
<td>48</td>
<td>0.88</td>
<td>50</td>
<td>1.00</td>
</tr>
</tbody>
</table>
Figure 22: As $k$, or the steepness of the probability of eviction curve, increases, the threshold of eviction (dashed line) approaches the level of subordinate help provided (solid line). The probability of eviction (red dotted line) remains near zero across all values of $k$. 
Figure 23: The level of subordinate helping (solid line) and the threshold of eviction (dashed line), as it varies with the immediate cost of eviction to subordinates (pink) and dominants (blue).