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RESEARCH ARTICLE

Behavioral and physiological evidence that increasing group size ameliorates the impacts of social disturbance

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ABSTRACT

Intra-group social stability is important for the long-term productivity and health of social organisms. We evaluated the effect of group size on group stability in the face of repeated social perturbations using a cooperatively breeding fish, *Neolamprologus pulcher*. In a laboratory study, we compared both the social and physiological responses of individuals from small versus large groups to the repeated removal and replacement of the most dominant group member (the breeder male), either with a new male (treatment condition) or with the same male (control condition). Individuals living in large groups were overall more resistant to instability but were seemingly slower to recover from perturbation. Members of small groups were more vulnerable to instability but recovered faster. Breeder females in smaller groups also showed greater physiological preparedness for instability following social perturbations. In sum, we discover both behavioral and physiological evidence that living in larger groups helps to dampen the impacts of social instability in this system.

KEY WORDS: Complex system, Cooperation, Dominance hierarchy, Social perturbation, Social scaling, Sociality

INTRODUCTION

Living in groups has various costs and benefits. For instance, group living can increase foraging efficiency (Berger, 1978), decrease predation risk (Foster and Treherne, 1981) and increase collective reproductive output (Modlmeier et al., 2012). In contrast, living in groups can sometimes decrease average per capita reproductive output (Bilde et al., 2007), promote disease transmission (Kappeler et al., 2015) and increase competition for food (Symington, 1988). For group living to evolve, the weight of the combined benefits of grouping must exceed the costs, and any factor that maximizes benefits whilst minimizing the costs of living in groups should promote the evolution of group-living and help to optimize sociality once it has evolved.

Social stability is one factor thought to help maximize benefits while minimizing the costs of group living. For instance, increased familiarity among group members can create a stabilizing effect

caused by increased predictability (Dall et al., 2004) and increase group productivity (Modlmeier et al., 2012; Pruitt and Riechert, 2011). Further, familiarity may be a mechanism for reducing within-group competition (Laskowski and Bell, 2013). Familiarity among groupmates can also enhance the effects of social buffering against environmental challenges (Hennessy et al., 2000; Livia Terranova et al., 1999) and decrease overall stress levels (Culbert et al., 2018; Kikusui et al., 2006; Nadler et al., 2016). Group stability also helps to reduce the costs of group living. For example, stable groups composed of familiar individuals experience less internal conflict, and so experience less stress from the threat of aggression or eviction (Pardon et al., 2004), reduced risk of injury, and waste fewer resources in competition (Marler et al., 1995). Even in non-cooperative territorial species, familiarity among neighbors commonly begets reduced aggression via dear enemy effects (e.g. Getty, 1987; Siracusa et al., 2017).

Despite the common finding that group stability helps to maximize group success, all groups in nature must endure some level of instability. Immigration/emigration, birth/death and alterations to dominance hierarchies, for example, result in alterations in group membership, and thus decrease within-group familiarity and stability. Many social species have therefore evolved mechanisms to help mitigate the negative impacts of such disruptive forces. For instance, some groups exhibit social rules that allow dominance hierarchies to swiftly reorganize following perturbation (Goldenberg et al., 2016). In other cases, reconciliatory communication mechanisms (e.g. specialized vocalization) aid in re-galvanizing damaged social bonds (Reddon et al., 2011; De Waal, 2000) and even particular individuals can help to dampen the negative impacts of group instability (Flack et al., 2005, 2006; McCowan et al., 2011). The traits that enable groups to dampen the acute impacts of social instability and to resume their former predictable states swiftly are important, because (1) stabilizing traits are potentially important targets for selection and (2) forces that compromise these traits risk imperiling the integrity and function of the social system.

Here, we examined how one group trait, group size, impacts the acute behavioral and physiological responses of group members to social disturbances and recoverability from them. We elected to focus on group size because it is known to mediate many costs and benefits associated with group living (Avilés and Tufiño, 1998), and because natural groups vary considerably in their size, with profound impacts on social selection (Brown et al., 2016). We predicted that living in large groups would diminish the acute impacts of social perturbations and increase group recoverability by distributing the negative impacts of social disturbance (e.g. acts of aggression) across more individuals. Larger groups may also recover more swiftly via enhanced affiliative behavior following social perturbations. We term this the 'distributed perturbation hypothesis' here. Alternatively, living in larger groups might increase the negative impacts of social perturbations (e.g. via

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increased aggression) or prevent groups from resuming quiescent behavioral states following disturbance. For instance, aggressive acts might initiate positive feedback fostering additional aggressive interactions in high-density environments and thus prevent groups from resuming their former stable states. We term this the 'aggressive feedback hypothesis'.

The impacts of social disturbances are likely to be evidenced physiologically as well. We therefore evaluated whether group size alters the degree to which group members are metabolically poised for intense bouts of acute or sustained physical activity following social perturbation. A higher capacity for intense activity might be necessary in preparation for, or as a training effect of, increased aggression. Many studies have identified links between various social behaviors and metabolic rates (see Huntingford et al., 2012 for review). However, reliance on oxygen consumption as a proxy for energy metabolism neglects the anaerobic processes that fuel burst-type movements typically associated with dominance behaviors (Plaut, 2001). Thus, a more refined focus on the biochemical pathways that underlie metabolic phenotypes should help elucidate links between physiology and behavior.

Enzymes are catalytic proteins that regulate biochemical reaction rates (Boyer and Krebs, 1986). Their expression is often plastic and can change in response to environmental stressors over a period of days to weeks (Beaman et al., 2016). Enzymes that catalyze regulatory steps of greater biochemical pathways can thus be plastically adjusted to meet an organism's peak metabolic demands in contrasting environments. Thus, *in vitro* measures of regulatory enzyme activities can represent upper thresholds for their respective pathways, and reflect the maximal capacity for these pathways to fuel peak activity *in vivo* (e.g. Vigelsø et al., 2014). Indeed, a number of studies have shown that activities of specific metabolic enzymes correlate strongly with intense social behaviors in a range of animal systems (AMP-activated protein kinase, Gilmour et al., 2017; lactate dehydrogenase, citrate synthase, cytochrome oxidase, Guderley, 2009; lactate dehydrogenase, citrate synthase, cytochrome oxidase, Guderley and Couture, 2005; citrate synthase, Kasumovic and Seebacher, 2013; lactate dehydrogenase, pyruvate kinase, Le François et al., 2005; citrate synthase, Regan et al., 2015). In this study, we focused on a key regulatory glycolytic enzyme (lactate dehydrogenase, LDH) and a key regulatory oxidative enzyme (citrate synthase, CS) that have been shown to reflect capacities for quick burst movements and more sustained aerobic activities, respectively (e.g. Alp et al., 1976; Childress and Somero, 1979; Johnston and Moon, 1981). We hypothesized that LDH and CS activities would scale with the most intense bouts of dominant actions displayed by an individual, and that these activities would be highest in individuals from destabilized groups.

To address these questions, we used the cooperative breeding cichlid *Neolamprologus pulcher*, endemic to Lake Tanganyika in the African Rift Valley. In the wild, groups usually comprise one dominant male–female breeding pair and one to 20 smaller, subordinate, non-breeding helpers (Balshine et al., 2001; Heg et al., 2005). Groups cooperate to care for the young of the dominant pair, maintain the group's territory, and defend the territory from both competitors and predators (Taborsky and Limberger, 1981; Wong and Balshine, 2011a). These fish also have a clear linear size-based dominance hierarchy, with increasing body size associated with increasing rank (Balshine-Earn et al., 1998). Natural groups regularly experience turnover of group members as helpers join or leave a group, or when group members perish (Heg et al., 2005; Stiver et al., 2004; Wong and Balshine, 2011b), with breeders estimated to be replaced a median of every 198–274 days (Dierkes

et al., 2005). Thus, this system provides a convenient evolutionary context to evaluate the impacts of group size on behavioral and metabolic responses to social instability and recoverability.

MATERIALS AND METHODS
Ethics
All experimental protocols were approved by the Animal Research Ethics Board of McMaster University (Animal Utilization Protocol no. 18-04-16), and were in compliance with the guidelines set by the Canadian Council on Animal Care (CCAC) regarding the use of animals in research.

Behavioral methods
Focal fish were haphazardly selected from a laboratory population containing approximately third-generation descendants of wild-caught *Neolamprologus pulcher* (Poll 1974) captured in 2014. Each social group was formed with a dominant pair (the largest male and female in each social group), and either four (small groups) or eight (large groups) subordinate helper fish. Thus there were a total of six fish in each 'small group' and 10 fish in each 'large group'. These group sizes reflect natural variation seen in the wild (Balshine et al., 2001; Heg et al., 2005). In total, we formed 12 small groups and 14 large groups. Small and large social groups were randomly allocated to either control (large, *n*=6; small, *n*=6) or treatment (large, *n*=8; small, *n*=6) conditions. Unbalanced group distribution was due to excessive aggression of some groups during the habituation period. To help reduce aggression and mortality, established breeding pairs were used. All helpers were unfamiliar to the dominant pair and had not previously cohabitated with them. Following group formation, the social groups were given 5 weeks to habituate and stabilize.

Each social group was maintained in a separate 189 liter aquarium containing two terracotta pot halves and two small PVC tubes (that served as both shelter and breeding substrate), two 10×10 cm mirrors, two sponge aeration filters, a heater and 3 cm deep coral sand as substrate. The mirrors served as a target of aggression to reduce within-group conflict. A water temperature of 27°C and 13 h:11 h light:dark photoperiod was maintained throughout the study. Each dominant male and female received an identifying dorsal fin clip, which has a minimal effect on behavior (Stiver et al., 2004). Fish were fed 6 days a week *ad libitum* with Nutrafin® basix large cichlid flakes.

The dominant male (standard length, SL: mean±s.e.m.=7.57± 0.92 cm) and dominant female (SL: 6.66±0.86 cm) were measured at the start of the experiment. To avoid confusion with later measures of dominance, these fish will subsequently be referred to as the breeder male and breeder female, though not all of these fish were observed breeding prior to the end of the experiment. The standard lengths of all helpers were estimated by an experienced observer (S.B.) (SL: mean=2.5 cm). Helper size was used only for group standardization purposes and not included in the analyses, and so estimation was used in place of physical measurement to reduce the need for further capture and minimize the stress experienced by the fish during group stabilization. In the treatment condition, the social perturbation consisted of the removal of the breeder male from one social group and replacing him with a new, unfamiliar breeder male. Breeder males were only exchanged with other males of the same group size. Therefore, breeder male fish in the treatment groups were swapped between tanks, and throughout the course of the experiment no treatment group experienced the same male twice. We ensured that the breeder males were always larger than the females, as is observed in the wild (Balshine et al., 2001; Desjardins et al., 2008; Wong et al., 2012). In

the control condition, the breeder male fish were removed from their tanks, handled for the same duration as the treatment males, but then returned to their home tank. This social disturbance procedure occurred twice (trial 1 and trial 2), with the manipulations conducted 1 week apart. All tanks were perturbed on the same day. Physiological Acclimation responses occur over a period of hours to weeks. Thus, as a conservative measure, perturbations were conducted twice to permit group members time to deploy morphological and enzymatic responses to reliably stable versus perturbed social conditions.

Behavioral observations were recorded using Canon VIXIA HF r-series cameras immediately before the manipulation, immediately following the manipulation, and then 4 and 24 h following the manipulation. Focal observation recordings were all 15 min long. The first 5 min of each recording were discarded to account for potential disturbance on remaining group members from capturing and returning the breeder male fish and human presence. All videos were scored by the same observer (H.M.A.), who was blind to treatment condition and time recording session. Behaviors were coded using McMaster University's Aquatic Behavioural Ecology Laboratory (ABEL) *N. pulcher* ethogram (Sopinka et al., 2009) and Behavioural Observation Research Interactive Software (BORIS) (Friard and Gamba, 2016). Behaviors were subdivided into the following categories: 'aggression' (chase, bite, ram, puffed throat, mouth-fighting, pseudo-mouth-fighting and head shake), 'submission' (submissive posture, submissive display, flee/chased and bitten) and 'affiliation' (soft touch, following and parallel swim).

We calculated a dominance index for each breeder male, breeder female, and for each group of helpers divided per capita, for each recording session. The dominance index is a well-established method for calculating dominance rank and is calculated as dominance index=(sum of aggressive acts given+sum of submissive acts received)–(sum of aggressive acts received+sum of submissive acts given) (Aubin-Horth et al., 2007). We also calculated an affiliation index for each breeder male and female, as well as a collective, per capita affiliation index across all helpers within a group, for each recording session, where affiliation index=sum of social acts given+sum of social acts received. We also recorded the observation period containing the highest dominance index score for breeder females in each tank, herein referred to as the maximum dominance index observed. Specifically, the maximum dominance index observed represents the dominance index of the time period with the highest levels of aggressive behavior in relation to submissive behaviors. This term therefore reflects what are presumably the most stressful and metabolically demanding moments we observed (Grantner and Taborsky, 1998).

The breeder female of each group was captured and rapidly (≤3 min) euthanized via overdose of benzocaine 2 days after the final perturbation; all breeder females were euthanized within a 5-h time frame. Females were measured and their heart, liver, and skeletal muscle just posterior to the dorsal fin were harvested and massed for further analyses. Breeder females were targeted for this analysis owing to their importance to the reproduction of the group, their high care activity and also their key position as both a breeder and a subordinate (Fitzpatrick et al., 2008).

Enzyme assays
In short, tissues were homogenized in 1:10 (m/v) homogenization buffer (0.1% Triton, 50 mmol l^{-1} Hepes, 1 mmol l^{-1} EDTA, pH 7.4; CAT: 100 mmol l^{-1} K phosphate buffer, 100 mmol l^{-1} KCl, 1 mmol l^{-1} EDTA, pH 7.4) on ice. Skeletal muscle homogenates were further diluted to 1:400 for the LDH activity

assay, whereas liver homogenates were diluted to 1:20 for both LDH and CS activity assays. Skeletal muscle homogenates were not further diluted for CS activity assays. All assays were run at 27°C in 96-well format on a Spectramax Plus 384 microplate reader (Molecular Devices, Sunnyvale, CA, USA). We used a wavelength of 340 nm to measure the disappearance of NADH (for LDH activity), and a wavelength of 412 nm to measure the production of 2-nitro-5-thiobenzoic acid (TNB; as a proxy of CS activity). For LDH and CS, extinction coefficients of 6.22 and 13.6 (mmol l^{-1})$^{-1}$ cm^{-1} were used, respectively.

Analyses and statistical methods
Dominance and affiliation indices were analyzed using a general linear mixed model (GLMM) fit by restricted maximum likelihood using the free and open software JAMOVI (Version 0.9, GAMLj module; https://www.jamovi.org). We fitted tank number and male ID as a random effect, and focal individual class (i.e. breeder female, breeder male, helpers), treatment type (i.e. control versus treatment), group size, trial number (i.e. trial 1 or trial 2) and time point (i.e. immediately before the manipulation, immediately after, 4 h after and 24 h after the manipulation) as fixed effects. We started with a maximal model and pruned non-significant terms (starting with high-order interactions) until we arrived at a model where all fixed effects were significant (Crawley, 2012; but see Harrison et al., 2018 for limitations to this approach). *Post hoc* analyses consisted of Bonferroni-corrected pairwise comparisons. We note here that with six to eight groups per treatment, our power to test higher-order interactions may be low. However, we have nonetheless opted for the statistical model that best reflects our experimental design.

To analyze the relationship between morphology (mass, relative heart mass, liver mass), maximum dominance index, and metabolic capacity (glycolytic and aerobic) across breeder females, we used general linear models (GLMs) fitted by ordinary least squares. For the maximum dominance index observed, we fitted treatment type and group size (factors), body mass, relative heart mass and liver mass (continuous covariates) as fixed effects. For metabolic capacity, LDH activity in either the skeletal muscle or the liver, or CS activity in either the skeletal muscle or the liver represented the dependent variable. Treatment type, group size (factors), maximum dominance index observed, body mass and other enzyme activity levels (continuous covariates) were fitted as fixed effects. We used the maximum dominance index observed as a fixed effect because LDH and CS measures convey individuals' capacities for peak activity. Thus, in addition to generalized locomotor activity, these effects also likely determine maximum capacities for social activities (e.g. aggression, flight and dominance), rather than baseline averages. We again started with a maximal model and pruned non-significant terms (starting with high-order interactions) until we arrived at a model where all fixed effects were significant. As a *post hoc* approach to test whether the effects of maximum dominance on enzyme activities were a potential effect of activity levels, we fitted respective models using mean activity measures as a covariate in place of maximum dominance. For all statistical tests, we used a significance threshold of α=0.05.

RESULTS
Behavioral responses
We detected a significant four-way interaction between class, treatment type, group size and time point on individuals' dominance scores (Table 1, Fig. 1A–D). We note again that our relatively limited power to test this effect means that results should be interpreted with caution. Consideration of the effect size (Table 1) and

Table 1. Statistical parameters for final (minimal) GLMM for dominance and affiliation indices

	Fixed factor	F	Numerator d.f.	Denominator d.f.	P
Dominance	**Treatment**	**5.431**	**1**	**21**	**0.030**
	Time point	1.137	3	552	0.334
	Group size	**8.089**	**1**	**22**	**0.009**
	Class	**146.421**	**2**	**552**	**<0.001**
	Treatment×Time point	0.440	3	552	0.724
	Treatment×Group size	**4.746**	**1**	**22**	**0.040**
	Time point×Group size	0.514	3	552	0.673
	Treatment×Class	**31.269**	**2**	**552**	**<0.001**
	Time point×Class	**3.944**	**6**	**552**	**<0.001**
	Group size×Class	**4.463**	**2**	**552**	**0.012**
	Treatment×Time point×Group size	0.527	3	552	0.664
	Treatment×Time point×Class	**3.809**	**6**	**552**	**<0.001**
	Treatment×Group size×Class	2.242	2	552	0.107
	Time point×Group size×Class	**5.069**	**6**	**552**	**<0.001**
	Treatment×Hours×Group size×Class	**3.686**	**6**	**552**	**0.001**
Affiliation	Group Size	0.004	1	22	0.947
	Treatment	3.112	1	22	0.092
	Trial number	**11.446**	**1**	**580**	**<0.001**
	Class	**8.027**	**2**	**580**	**<0.001**
	Time point	1.985	3	580	0.115
	Group Size×Treatment	1.248	1	22	0.276
	Treatment×Time point	1.758	3	580	0.154
	Trial number×Time point	**5.451**	**3**	**580**	**0.001**
	Group size×Time point	1.196	3	580	0.310
	Group size×Treatment×Time point	**2.749**	**3**	**580**	**0.042**

Class of individual (i.e. female, male, helpers), treatment type (i.e. control versus treatment), group size, trial number (i.e. trial 1 or trial 2), and time point (i.e. immediately before the manipulation, immediately after, 4 h after and 24 h after the manipulation). Bold indicates $P<0.05$.

examination of the figures (Fig. 1A–D) gives a guide as to the biological importance of this term. In control tanks housing small groups, breeder male dominance was consistently more than five-fold greater than that of breeder females, although this trend was significant only immediately after the control perturbation (Fig. 1A; Table S1 for pairwise comparisons). In control tanks housing large groups, there were no significant differences in dominance between the breeder males, breeder females and helpers, although the helpers consistently
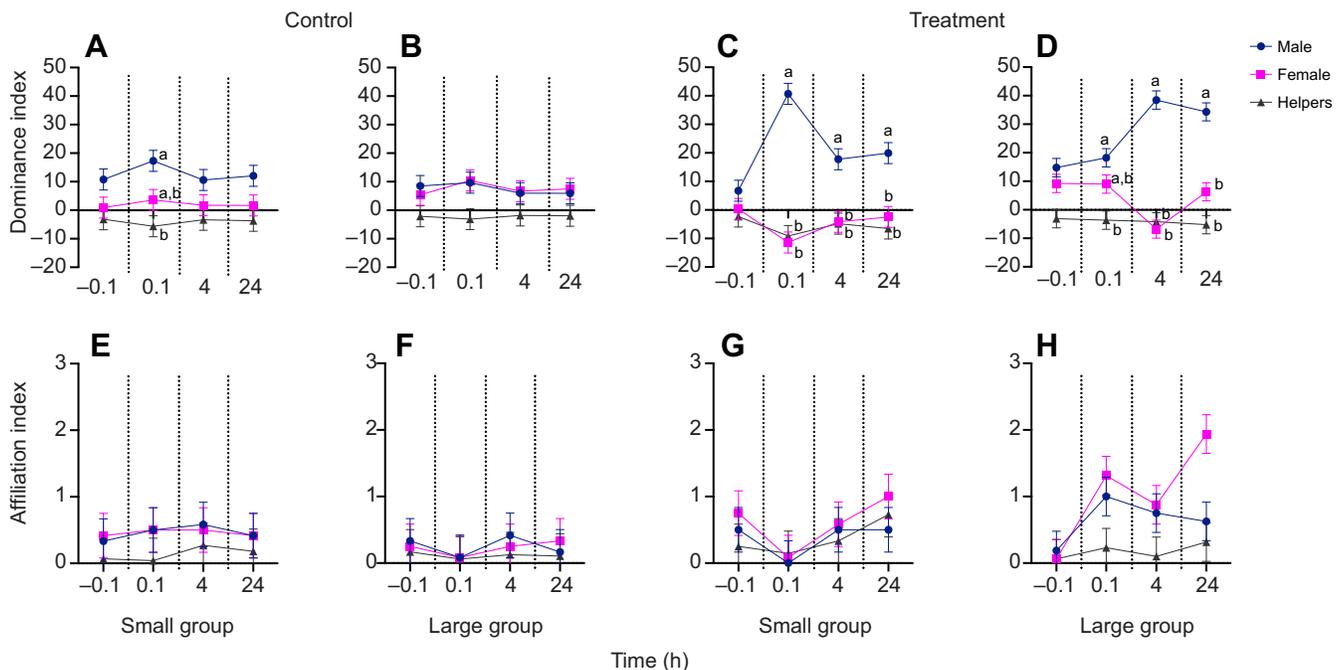
Fig. 1. Time courses for mean dominance and affiliation indices. (A–D) Mean dominance indices in males (blue), females (pink) and helpers (gray) from small and large control (A and B, respectively) and treatment (C and D, respectively) groups. (E–H) Mean affiliation indices from small and large control (E and F, respectively) and treatment (G and H, respectively) groups. Different letters represent significant ($P<0.05$) differences between males, females and helpers within each respective time point, as determined by *post hoc* comparisons.

had a five-fold lower dominance score than both the breeder males and breeder females (Fig. 1B; Table S1). These results suggest that male aggression is more pronounced in small control groups and that breeder females display more submissive acts in response.

In treatment tanks housing small groups, we found that the dominance indices of the breeder females were significantly lower than those of the breeder males at all time points, especially immediately following the perturbation (Fig. 1C; Table S1). However, in treatment tanks housing large groups, there was a delayed spike in breeder male dominance relative to breeder females, in that no significant difference in dominance between breeder males and females was apparent until 4 h after the perturbation (Fig. 1D; Table S1). Helper dominance remained significantly lower than breeder male dominance in treatment groups across all time points and for both group sizes. There was no significant effect of trial number (i.e. perturbation 1 versus perturbation 2) in any of the analyses.

There was a significant interaction term between group size, treatment and time point on social affiliation scores. We further detected a significant interaction term between trial number and time point, and a main effect of individuals' class (breeder female, breeder male, helper) on social affiliation scores (Table 1, Fig. 1E–H). Although there was no effect of group size on affiliation scores in the control groups, affiliation conspicuously increased following perturbation in the large treatment groups relative to the small treatment groups. Groups gradually increased affiliative behaviors following the introduction of a new breeder male, but somewhat decreased affiliative behavior following the introduction of a second new breeder male (Table 1; Table S2 for pairwise comparisons). Finally, breeder females had the highest affiliation index followed by breeder males, and then by helpers in the treatment groups (Fig. 1E–H, Table 1; Table S2 for pairwise comparisons).

Morphometric and enzyme responses
We found an interaction between body mass and group size on the maximum dominance index observed (Table 2, Fig. 2A). Here, maximum scores for dominance increased with breeder female body size in small groups and decreased with body size in large groups. There were no significant effects of heart tissue in relation to body mass; however, there was a significant interaction between treatment and group size on relative liver size (hepatosomatic index, HSI; Table 2, Fig. 2B). Specifically, the mean HSI was elevated in breeder females of small groups in control tanks and large groups in treatment tanks.

There was a significant interaction between the dominance indices of breeder females and treatment on liver LDH activity (Table 2, Fig. 2C). Liver LDH activity scaled negatively with breeder female dominance in control groups, and positively with breeder female dominance in treatment groups. This result suggests that our social perturbation treatment was successful in priming breeder females to be more enzymatically prepared for sudden bursts of activity. We found no significant effects of dominance on muscle LDH activity, or liver and skeletal muscle CS activity (Table 2). *Post hoc*, we found no significant effects of mean level of breeder female activity on liver LDH activities (see Dataset 1 for the liver model, which is identical to Table 2 but with 'mean activity' replacing 'maximum dominance' as a covariate), suggesting that maximum dominance affects glycolytic capacity independently from greater levels of general locomotor activity.

DISCUSSION
Group stability tends to increase the benefits and decrease the costs of social living (Berger, 1978; Modlmeier et al., 2012; Pruitt and

Table 2. Statistical parameters for final (minimal) GLM for female-level effects

	SS	d.f.	F	P
Morphometrics on max. dominance				
Body mass	247	1	1.38	0.059
Group size	621	1	3.48	0.075
Group size×Body mass	**866**	**1**	**4.85**	**0.038**
Residuals	5307	22		
Relative liver size (HSI)				
Group size	0.007	1	0.04	0.846
Treatment	0.105	1	0.54	0.469
Group size×Treatment	0.831	1	4.31	0.050
Residuals	4.239	22		
Liver LDH				
Body mass	0.174	1	0.73	0.402
Treatment	0.238	1	1.00	0.329
Max. dominance	0.212	1	0.89	0.357
Muscle LDH	**1.094**	**1**	**4.61**	**0.045**
Treatment×Max. dominance	**1.732**	**1**	**7.30**	**0.014**
Residuals	4.541	19		
Muscle LDH				
Body mass	154	1	1.647	0.212
Residuals	2256	24		
Liver CS				
Body mass	0.004	1	0.184	0.672
Residuals	0.531	22		
Muscle CS				
Body mass	0.300	1	0.211	0.650
Residuals	34.19	24		

CS, citrate synthase; HSI, hepatosomatic index; LDH, lactate dehydrogenase. Max. dominance is the maximum dominance index observed. Bold means $P<0.05$.

Riechert, 2011), and groups often exhibit mechanisms to return to a stable state following disturbance (Goldenberg et al., 2016; McCowan et al., 2011; De Waal, 2000). We sought to determine the effects of group size on the group's ability to return to social homeostasis in the face of a repeated social stressor. Specifically, we hypothesized a large group would either reduce overall aggression, through the distributed perturbation hypothesis, or increase and sustain overall aggression, through the aggressive feedback hypothesis. Here, we found more support for the distributed perturbation hypothesis, though additional moderating forces are also likely at play.

Small groups showed more disparate dominance indices between the most dominant fish (breeder males) and the subordinate fish (breeder females and helpers). This is most obvious when comparing the control groups (Fig. 1A,B). Previous work has found large groups benefitting from larger territories with increased shelter and more opportunities to feed (Balshine et al., 2001). Our results further imply that small groups may be inherently more polarized (and less stable) than large groups, even when social conditions remain relatively steady. In other words, large groups likely benefit from both material and non-material social advantages. The timing of dominance index spikes varied with group size in our treatment groups: in small groups, changes to and inequality of dominance indices appeared immediately following the perturbation (Fig. 1C), while in large groups change in the indices lagged following perturbation (Fig. 1D). Small groups also appear to return to baseline states faster, as observed in the apparent reduction in breeder male dominance 24 h following the perturbations, while the dominance of large group breeder males remain elevated. Together, these results suggest that large groups are more resistant to social state change and/or that state change in
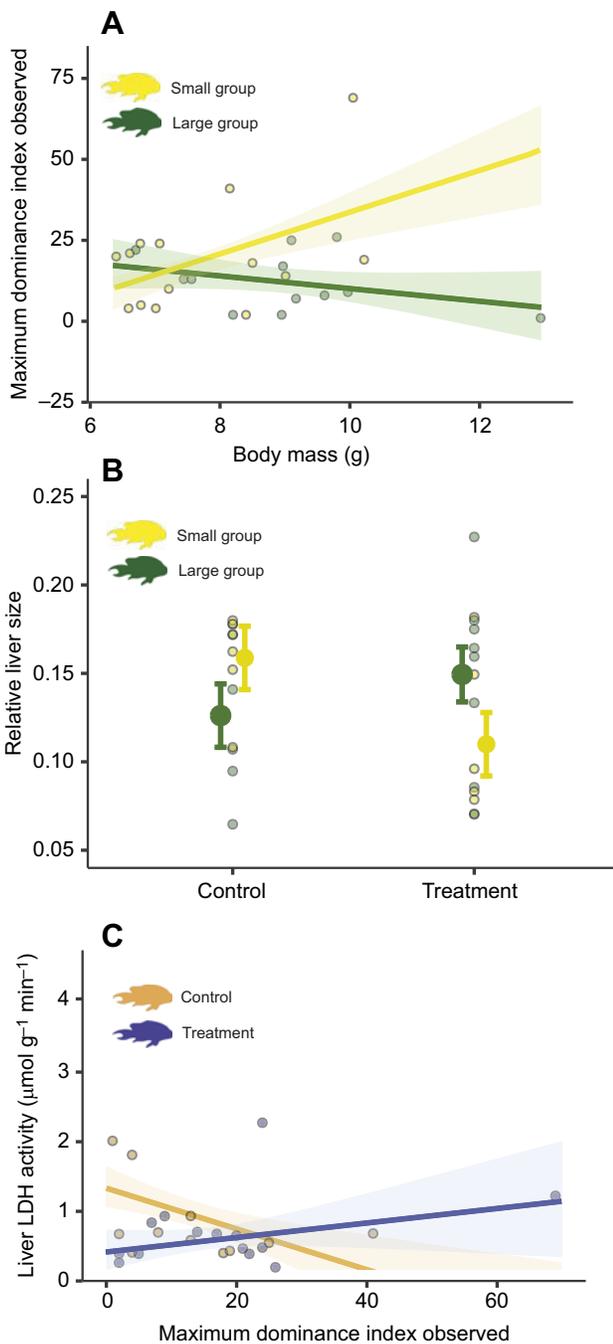
Fig. 2. Relationships between behavioral, morphometric and enzymatic traits. Maximum dominance index observed scales with body mass depending on group size (A), hepatosomatic index (HSI) changes as a function of group size depending on treatment type (B), and liver LDH activity scales with maximum dominance index observed depending on treatment type (C). Small and large groups (A,B) are represented by yellow and green, respectively, whereas treatment and control groups (C) are represented by violet and orange, respectively. Enclosed circles represent observed scores. Note, the directionality and patterns of the relationship remain when we remove the two most extreme data points.

large groups is slower than in small groups. This could be because new breeder males delay asserting their dominance in larger groups until they have had time to evaluate their new social setting and potential competitors. Regardless of the mechanism, this conveys

that larger groups might offer their constituents buffering effects against ephemeral social perturbations in a manner that small groups do not.

Additional circumstantial evidence from affiliation indices and body mass hint that smaller groups are more stressful social environments following perturbation. One can observe an increase in the affiliative behaviors of breeder males and especially breeder females following social perturbations in large groups (Fig. 1H). This conveys that the new breeder pair begins establishing a social bond in these groups. If this happens in small groups too, then it is certainly less conspicuous (Fig. 1G). We further note that large breeder females exhibited higher dominance in small groups, irrespective of control versus treatment, whereas no relationship between body size and dominance was observed in large social groups. This group-size-dependent relationship conveys that more acts of dominance transpire in small groups occupied by large breeder females, whereas the dominance indices of breeder females in large groups are near uniformly low (Fig. 2A). This lack of variation in large groups provides further evidence that large social groups are less volatile and more stable social environments than small groups. In *N. pulcher*, the strength of social buffering is largely managed by aggression rates (Culbert et al., 2019), so the decreased aggression found in these large groups might facilitate recovery from social perturbation.

The significant interaction of treatment type and group size on HSI further reveals that social disturbance affects breeder female physiology as a factor of group size. Decreased liver size in treated females from small groups and enhanced liver size in treated females from large groups may indicate changes in metabolic energy demands, short-term nutritional status, growth rates, synthesis of vitellogenin and eggshell proteins, or a combination thereof (e.g. Adams and McLean, 1985; Berg et al., 2004; Everaarts et al., 1993; Korsgaard et al., 1986; Larsen et al., 1992). Although we lack the resolution to disentangle the mechanisms affecting treatment- and group-size-driven differences in HSI here, higher HSI has been linked to increased dominance in sticklebacks (Guderley and Couture, 2005). Furthermore, concurrent changes in liver LDH activities can indicate that metabolic energy demands, at least in part, contribute to this effect. Specifically, we show that treatment type directionally mediates the relationship between dominance and glycolytic capacity.

The divergent relationship between dominance and LDH activity provides evidence that our social perturbations were successful in instigating an enzymatic response in breeder females. Liver LDH activity increased with breeder female dominance in treatment groups, which were characterized by the largest gaps in dominance between breeder males and females. This further suggests that breeder female dominance increases metabolic preparedness for aggression in these groups relative to controls. By contrast, in the control condition, LDH activity levels decreased with breeder female dominance, suggesting that greater dominance is associated with reduced glycolytic capacity and potentially greater stability in these groups. Because the control perturbation was characterized by a familiar breeder male, we suggest that pre-established social relationships dampen the aggressive actions that foster glycolytic capacity. Whether these phenotypic differences reflect a regulated response to social stress, a positive feedback effect of training, or a combination of the two, remains to be examined. However, the lack of relationship between liver LDH activity and greater breeder female activity levels suggests that these trends are not simply a feedback effect of exercise training.

Overall, we found more support for the distributed perturbation hypothesis from both behavioral and physiological indicators. Physiologically, breeder females elevated their glycolytic capacity in small groups and when faced with strong social perturbations (treatment). Behaviorally, small groups also showed a larger difference in dominance indices across group members, whereas in large groups, dominance indices were slower to polarize following a perturbation and were associated with a surge of affiliative behaviors as well, both observations circumstantially supporting the distributed perturbation hypothesis. In contrast, the gap in dominance indices shrunk faster following the perturbation in small groups compared with large groups, potentially supporting the aggressive feedback hypothesis. It therefore appears that different group sizes create different responses to the forces of instability: small groups experience larger instability following a social perturbation but recover more rapidly and appear physiologically primed for more instability, whereas large groups are more resistant to the instability of perturbation but appear to recover more slowly. In aggregate, these results convey that the demographic traits of social groups can play a large role in shaping group susceptibility to and recoverability from social disturbance, and that larger groups could exhibit greater levels of social stability and social inertia.

Author contributions
Conceptualization: H.M.A., A.G.L., D.N.F., B.L.M., B.M.C., S.B., J.N.P.; Methodology: H.M.A., A.G.L., D.N.F., B.L.M., B.M.C., S.B., J.N.P.; Formal analysis: A.G.L., J.N.P.; Investigation: H.M.A., A.G.L., D.N.F., B.L.M., B.M.C., S.B.; Resources: S.B., J.N.P.; Data curation: H.M.A., A.G.L.; Writing - original draft: H.M.A., A.G.L., J.N.P.; Writing - review & editing: H.M.A., A.G.L., D.N.F., B.L.M., B.M.C., S.B., J.N.P.; Visualization: A.G.L.; Supervision: H.M.A., A.G.L., B.M.C., S.B., J.N.P.; Project administration: H.M.A., A.G.L., S.B., J.N.P.; Funding acquisition: S.B., J.N.P.

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