BEHAVIORAL AND PHYSIOLOGICAL EVIDENCE THAT INCREASING GROUP SIZE AMELIORATES THE IMPACTS OF SOCIAL DISTURBANCE

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SUMMARY STATEMENT

Social stability is vital for group productivity and long-term persistence. Here, both behavioral and physiological evidence conveys that larger groups are less susceptible to social disturbance.

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). Individuals living in large groups were overall more resistant to instability but were seemingly slower to recover from perturbation. Members of small group 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 recover both behavioral and physiological evidence that living in larger groups helps to dampen the impacts of social instability in this system.

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.

One factor thought to help maximize the cost/benefit ratio of group living is social stability. For instance, increased familiarity among group members can allow for increased social niche specialization (Laskowski and Pruitt, 2014), reduced within-group competition (Laskowski and Pruitt, 2014), and increased group productivity (Modlmeier et al., 2012). Familiarity of 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, Walsberg, White, Moore, & Marler, 1995; Jordan et al 2010). 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 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 (Waal, 2000; Reddon et al 2019), and even particular individuals can help to dampen the negative impacts of group

instability (Flack et al., 2005; Flack et al., 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 *i*) stabilizing traits are potentially important targets for selection and *ii*) 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 Tufino, 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 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, Tamilselvan, & Jenjan, 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 behaviour.

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 maximum capacity for these pathways to fuel peak activity *in vivo* (e.g.Vigelsø, Andersen, & Dela, 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 (Gilmour et al., 2017; Guderley, 2009; Guderley and Couture, 2005; Kasumovic and Seebacher, 2013; Le François et al., 2005; 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, Newsholme, & Zammit, 1976; Childress & Somero, 1979; Johnston & 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 use the cooperative breeding cichlid *Neolamprologus pulcher*, endemic to Lake Tanganyika in the African Rift Valley. In the wild groups are usually comprised of one dominant male-female breeding pair and 1-20 smaller, subordinate, non-breeding helpers (Balshine et al., 2001; Heg et al., 2019). 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 & Limberger, 1981; Wong & 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 in group members as helpers join or leave a group, or when group members perish (Stiver et al 2006; 2007; Heg et al., 2019; Wong & Balshine, 2011b). 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.

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 lab population containing descendants of wild-caught *N. pulcher* captured in 2014. Large and small groups were formed with a dominant pair (the largest male and female in each social group), and either four ("small groups", n=12) or eight ("large groups", n=14) subordinate helper fish. To reduce aggression and mortality, dominant pairs were taken from pre-existing breeding pairs. All helpers were unfamiliar to the dominant pair and had not previously cohabitated with them. Following group formation, the social groups were allowed to habituate and stabilize for five weeks.

Each social group was maintained in separate, 189 L aquariums containing two terracotta pot halves and two small PVC tubes (that served as both shelter and breeding substrate), two 10 cm x 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 morbidity from within-group conflict. A water temperature of 27° C and 13:11 light to dark hour 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 six days a week ad libitum with Nutrafin® basix large cichlid flakes.

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. The dominant male (standard length, SL: average=7.57 cm, SEM=0.92) and dominant female (SL: average=6.66 cm, SEM=0.86) were measured at the start of the experiment. To avoid confusion with later measures, these fish will subsequently be referred to as the *breeding male* and *breeding female*. The standard lengths of all helpers were estimated by an experienced observer (SB) (SL: average=2.52 cm, SEM=0.07). In the treatment condition, the social perturbation consisted of the removal of the breeding male from one social group and replacing him with a new, unfamiliar breeding male that previously dwelled in another social group of identical size, tank set up, and

group composition. Therefore, breeding male fish in the treatment groups were swapped between tanks. We ensured that the breeding males were always larger than the females, as observed in the wild (Balshine et al., 2001; Desjardins et al., 2008; Wong et al., 2012)In the control condition, the breeding 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 one week apart. All tanks were perturbed on the same day. Perturbations were conducted twice to permit group members time to deploy an enzymatic response to reliably stable vs. perturbed social conditions.

Behavioral observations were recorded using Canon VIXIA HF r-series cameras immediately before the manipulation, immediately following the manipulation, and then four, and twenty-four hours following the manipulation. In addition, two baselines were recorded twenty-four and forty-eight hours prior to the first manipulation. Focal observation recordings were all fifteen minutes long. The first five minutes of each recording were discarded to account for potential disturbance on remaining group members from capturing and returning the dominant male fish and human presence. All videos were scored by the same observer (HA), who was blind to treatment condition and time step. Behaviors were coded using McMaster University's Animal Behavior Ecology Laboratory (ABEL) *N. pulcher* ethogram (Sopinka et al., 2009) and Behavioral Observation Research Interactive Software (BORIS) (Friard and Gamba, 2016). Behaviors were subdivided into the 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 (DI) for each breeding male, breeding female, and for each group of helpers divided per capita, for each recording session. The DI is a well-established method for calculating dominance rank and = (sum of aggressive acts given + sum of submissive acts received) - (aggressive acts received + submissive acts given). We calculated an affiliation index for each breeding male, breeding female, and for each group of helpers divided per capita, for each recording session, where affiliation rank and = (sum of social acts given + sum of social acts received). We also recorded the most dominant time step for breeding females in each tank, herein referred to as the *maximum dominance index observed*. Specifically, the maximum dominance index observed represents the DI of the time period with the highest levels of

aggressive and submissive behaviors. This term therefore reflects what are presumably the most stressful and metabolically demanding moments we observed (Grantner and Taborsky, 1998).

The breeding female of each group was captured and rapidly (≤3 minutes) euthanized via overdose of benzocaine within forty-eight hours of the final perturbation. Females were measured and their skeletal muscle just posterior to the dorsal fin, heart, and liver were harvested and massed for further analyses.

Enzyme Assays

In short, tissues were homogenized in 1:10 (m/v) homogenization buffer (0.1% Triton, 50 mM Hepes, 1mM EDTA, pH 7.4; CAT: 100 mM K phosphate buffer, 100 mM KCl, 1 mM 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). 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). Extinction coefficients of 6.22 mM^{-1} cm^{-1} and 13.6 mM^{-1} cm^{-1} were used for LDH and CS, respectively.

Analyses and Statistical Methods

Dominance and affiliation indices were analyzed using a mixed linear model (GLMM) fit by REML using the free and open software JAMOVI (Version 0.9; GAMLj module; https://www.jamovi.org). We fitted tank number as a random effect, and focal individual class (i.e. female, male, helpers), treatment type (i.e. control vs. treatment), group size, trial number (i.e. trial 1 or trial 2), and timepoint (i.e. immediately before the manipulation, immediately after, four hours after and twenty-four hours after the manipulation) as fixed effects. We started with a saturated model and pruned non-significant terms (starting with high-order interactions) until we arrived at a model where all fixed effects were significant. *Post-hoc* analyses consisted of Bonferroni-corrected pairwise comparisons.

To analyze the relationship between body traits (mass, relative heart mass, liver mass) and the maximum dominance index observed during the experiment on metabolic capacity

(glycolytic and aerobic) across females, we used general linear models (GLM) fit by OLS. For the maximum dominance index observed, we fitted treatment type and group size (factors) and body mass, relative heart mass, and liver mass (continuous covariates) as fixed effects. For metabolic capacity, LDH activity in either the muscle or the liver, or CS activity in either the 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 saturated 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 $\alpha = 0.05$.

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). In control tanks housing small groups, male dominance was consistently more than five-fold greater that of females, although this trend was significant only immediately after the control perturbation (Fig. 1A; S1 for pairwise comparisons). In control tanks housing large groups, there were no significant differences in dominance between the males, females, and helpers; although the helpers consistently had a five-fold lower dominance scores than both the males and females (Fig. 1B; S1). These results suggest that male aggression is more pronounced in small control groups and that females display more submissive acts in response.

In treatment tanks housing small groups, we found that the dominance indices of the females were significantly lower than that of the males at all time points, especially immediately following the perturbation (Fig. 1C; S1). However, in treatment tanks housing large groups there

was a delayed spike in male dominance relative to females, where no significant difference in dominance between males and females was apparent until four-hours after the perturbation (Fig. 1D; S1). As expected, helper dominance remained significantly lower than male dominance across all time points in both group sizes. There was no significant effect of trial number (i.e. perturbation 1 vs. 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 (female, male, helper) on social affiliation scores (Table 1; Fig. 1E-H). While 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 male, but somewhat decreased affiliative behavior following the introduction of a second new male (Table 1; see appendix for pairwise comparisons). Finally, females had the highest affiliation index followed by males, and then by helpers in the treatment groups (Fig. 1E-H; Table 1; S2 for pairwise comparisons).

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 female body size in small groups and decreased with body size in large groups. There were no significant effects of relative heart or liver size on maximum scores for dominance, and no main effect of treatment type (i.e. control vs. treatment).

Enzyme responses

There was a significant interaction between female dominance and group size on muscle LDH activity (Table 2; Fig. 2B). Muscle LDH activity scaled positively with dominance in small groups and it scaled negatively with dominance in large groups, suggesting that breeding females were more poised for intense bursts of activity in smaller groups. We also found that both liver and skeletal muscle LDH activities scaled negatively with female dominance in control groups, and positively with female dominance in treatment groups (Table 2; Fig. 2C, D). These results convey that our social perturbation treatment was successful in causing the breeding females to be enzymatically prepared for sudden bursts of activity. We found no significant effects of dominance on liver or muscle CS activity (Table 2), which is associated with endurance

activities and aerobic metabolism. Instead, the anaerobic component of metabolism as captured by LDH activity was more responsive to our social perturbations. *Post-hoc*, we found no significant effects of mean level of female activity on liver or skeletal muscle LDH activities (appendix 4), 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; Laskowski and Pruitt, 2014; Modlmeier et al., 2012), and groups often exhibit mechanisms to return to a stable state following disturbance (Goldenberg et al., 2016; McCowan et al., 2011; 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 (breeding males) and the subordinate fish (breeding females and helpers). This is most obvious when comparing the control groups (Fig. 1A,B). Previous studies have found a positive relationship between group size and long-term group survival (Heg et al., 2019), with large groups benefitting from higher quality territories and more opportunities to feed (Balshine et al., 2001). These latest 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 slide back towards baseline states faster, as observed in the apparent reduction in breeding male dominance twenty-four hours following the perturbations, while the dominance of large group males remain elevated. Together, these results

311 suggest that large groups are more resistant to social state change and/or that state change in
312 large groups is slower than in small groups. This could be because new males delay asserting
313 their dominance in larger groups until they have had time to evaluate their new social setting and
314 potential competitors. Regardless of the mechanism, this conveys that larger groups might offer
315 their constituents buffering effects against ephemeral social perturbations in a manner small
316 groups do not.
317 Additional circumstantial evidence from affiliation indices and body mass hint that
318 smaller groups are more stressful social environments following perturbation. One can observe
319 an increase in the affiliative behaviors of males and especially females following social
320 perturbations in large groups (Fig. 1H). This conveys that the new breeding pair begins
321 establishing a social bond in these groups. If this happens in small groups too, then it is certainly
322 less conspicuous (Fig. 1G). We further note that large females exhibit higher dominance in small
323 groups, irrespective of control vs. treatment, whereas no relationship between body size and
324 dominance was observed in large social groups. This group-size dependent relationship conveys
325 that more volatile acts of dominance transpire in small groups occupied by large females,
326 whereas the dominance indices of females in large groups are near uniformly low (Fig. 2A).
327 This lack of variation in large groups provides further evidence that large social groups are less
328 volatile and more stable social environments than small groups. In *N. pulcher* the strength of
329 social buffering is largely managed by aggression rates (Culbert et al., 2019), so the decreased
330 aggression found in these large groups might facilitate recovery from social perturbation.
331 Elevated LDH activities in muscle and liver suggest enhanced glycolytic preparedness and
332 capacity for the powerful burst movements that characterize aggressive acts (Le François et al.,
333 2005). In Arctic charr (*Salvelinus alpinus*), for instance, fast-twitch muscle fibers of dominant
334 individuals possess LDH activities more than 15% greater than their subordinate counterparts
335 (Le François et al., 2005). Our work, however, shows that group size directionally mediates the
336 relationship between dominance and glycolytic capacity. LDH activity was highest in the most
337 dominant animals but only in small groups, which also have the most disparate dominance
338 indices between males and females (Fig. 1A, C). In large groups however, more dominant
339 females were characterized by lower LDH activity levels. These trends suggest that the more
340 dominant females in small groups must be better primed to perform (or potentially avoid)
341 aggressive actions, while the more dominant females in large groups are not. Whether these

342 phenotypic differences reflect a regulated response to social stress, a positive feedback effect of
343 training, or a combination of the two, remains to be examined. However, the lack of relationship
344 between these enzyme measures and greater female activity levels suggests these trends are not
345 simply a feedback effect of exercise training. Together, our findings suggest that breeding
346 females in small groups experience greater instability following disturbance and are
347 metabolically prepared for more instability.

348 The divergent relationship between dominance and LDH activity provides evidence that
349 our social perturbations were successful in instigating an enzymatic response in females. Muscle
350 and liver LDH activities increased with female dominance in treatment groups, which were
351 characterized by the largest gaps in dominance between males and females. This further suggests
352 that female dominance increases metabolic preparedness for aggression in these groups relative
353 to controls. By contrast, in the control condition, LDH activity levels decreased with female
354 dominance, suggesting greater dominance is associated with reduced glycolytic capacity and
355 potentially greater stability in these groups. Because the control perturbation was characterized
356 by a familiar male, we suggest that preestablished social relationships dampen the aggressive
357 actions that foster glycolytic capacity.

358 Overall, we found more support for the *distributed perturbation hypothesis* from both
359 behavioral and physiological indicators. Physiologically, breeding females elevated their
360 glycolytic capacity in small groups and when faced with strong social perturbations (treatment).
361 Behaviorally, small groups also showed a larger difference in dominance indices across group
362 members, while in large groups' dominance indices were slower to polarize following a
363 perturbation and were associated with a surge of affiliative behaviors as well, both observations
364 circumstantially supporting the *distributed perturbation hypothesis*. On the other hand, the gap in
365 dominance indices shrunk faster following the perturbation in small groups compared to large,
366 potentially supporting the *aggressive feedback hypothesis*. It therefore appears that different
367 group sizes create different responses to the forces of instability: small groups experience larger
368 instability following a social perturbation, recover more rapidly but are physiologically primed
369 for more instability, whereas large groups are more resistant to the instability of perturbation but
370 appear to recover more slowly. In aggregate, these results convey that the demographic traits of
371 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.

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Competing Interests

No competing interests declared.

Data Availability

Behavioral data will be uploaded upon completion as supplementary.

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

Dominance	Fixed Factor	F	Num df	Den df	p
	Treatment	5.439	1	22	0.029
	Time point	1.137	3	554	0.334
	Group Size	8.138	1	22	0.009
	Class	146.405	2	554	< .001
	Treatment ✻ Time point	0.44	3	554	0.724
	Treatment ✻ Group Size	4.782	1	22	0.04
	Time point ✻ Group Size	0.514	3	554	0.673
	Treatment ✻ Class	31.266	2	554	< .001
	Time point ✻ Class	3.944	6	554	< .001
	Group Size ✻ Class	4.463	2	554	0.012
	Treatment ✻ Time point ✻ Group Size	0.527	3	554	0.664
	Treatment ✻ Time point ✻ Class	3.809	6	554	< .001
	Treatment ✻ Group Size ✻ Class	2.242	2	554	0.107
	Time point ✻ Group Size ✻ Class	5.069	6	554	< .001
	Treatment ✻ Hours ✻ Group Size ✻ Subject	3.685	6	554	0.001
Affiliation					
	Group Size	0.00442	1	22	0.948
	Treatment	3.12959	1	22	0.091
	Trial #	11.43825	1	580	< .001
	Class	8.02106	2	580	< .001
	Time point	1.98344	3	580	0.115
	Group Size ✻ Treatment	1.25136	1	22	0.275
	Treatment ✻ Time point	1.75654	3	580	0.154
	Trial # ✻ Time point	5.4469	3	580	0.001
	Group Size ✻ Time point	1.1949	3	580	0.311
	Group Size ✻ Treatment ✻ Time point	2.74729	3	580	0.042

517 Numerator degrees of freedom, Num df; Denominator degrees of freedom, Den df; Shading, $p < 0.05$.

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

Morphometrics on Max Dom	**SS**	**df**	**F**	**p**
Model	1501	3	2.5	0.086
BM	295	1	1.48	0.237
Group Size	636	1	3.18	0.088
Group Size ✻ BM	902	1	4.51	0.045
Residuals	4400	22		
Liver LDH				
Model	2.266	5	1.796	0.164
BM	0.154	1	0.612	0.444
Treatment	1.64	1	6.502	0.02
Max Dominance	0.203	1	0.803	0.382
Muscle LDH	1.115	1	4.419	0.05
Treatment ✻ Max Dominance	1.649	1	6.536	0.02
Residuals	4.541	18		
Muscle LDH				
Model	735.17	6	2.911	0.037
BM	79.48	1	1.888	0.186
Treatment	128.44	1	3.051	0.098
Max Dominance	13.16	1	0.313	0.583
Group Size	4.98	1	0.118	0.735
Group Size ✻ Max Dominance	244.01	1	5.797	0.027
Treatment ✻ Max Dominance	331.37	1	7.872	0.012
Residuals	757.69	18		
Liver CS				
Model	0.00445	1	0.184	0.672
BM	0.00445	1	0.184	0.672
Residuals	0.53122	22		
Muscle CS				
Model	0.3	1	0.211	0.65
BM	0.3	1	0.211	0.65
Residuals	34.19	24		

522 BM, body mass; Max Dominance, Maximum Dominance Index Observed; Shading, $p < 0.05$.

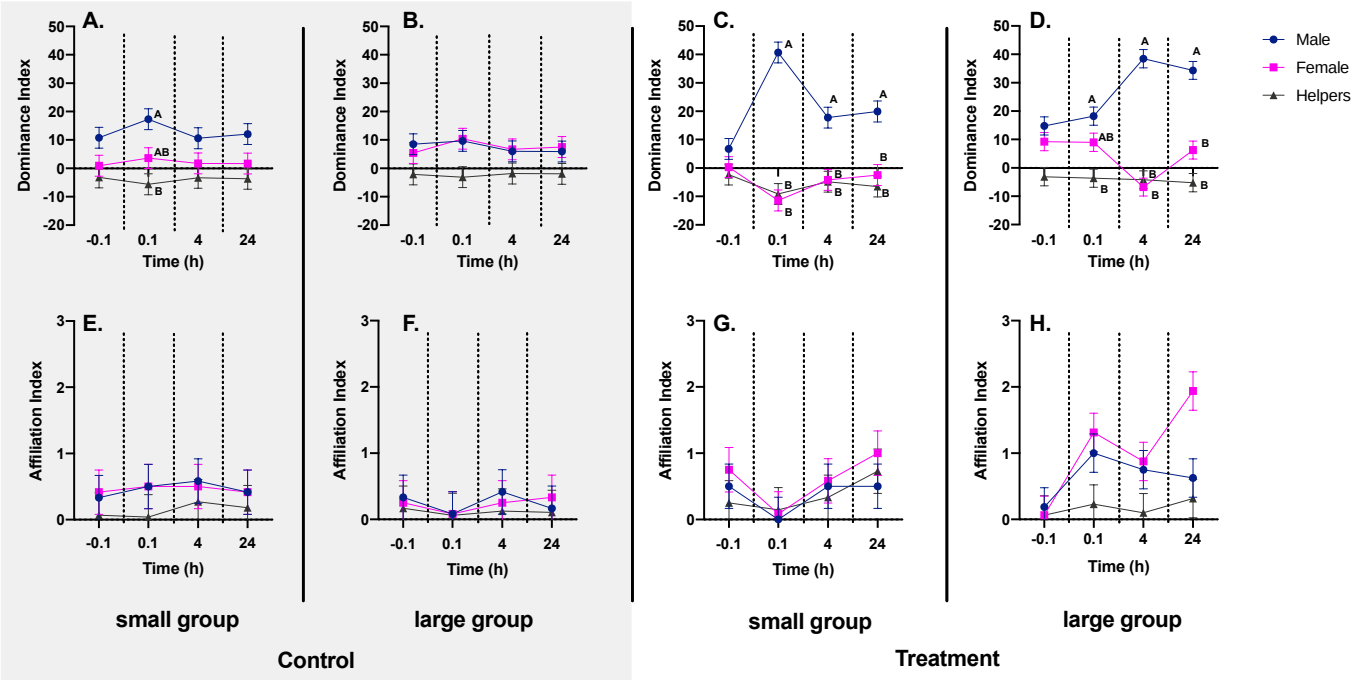

Figure 1. Time courses for mean dominance and affiliation indices. Mean dominance indices in males (blue), females (pink), and helpers (grey) from small and large control (A and B, respectively) and treatment (C and D, respectively) groups. Mean affiliation indices from small and large control (E and F, respectively) and treatment (G and H, respectively) groups. Different letters represent differences between males, females and helpers within each respective timepoint, as determined by *post hoc* comparisons.

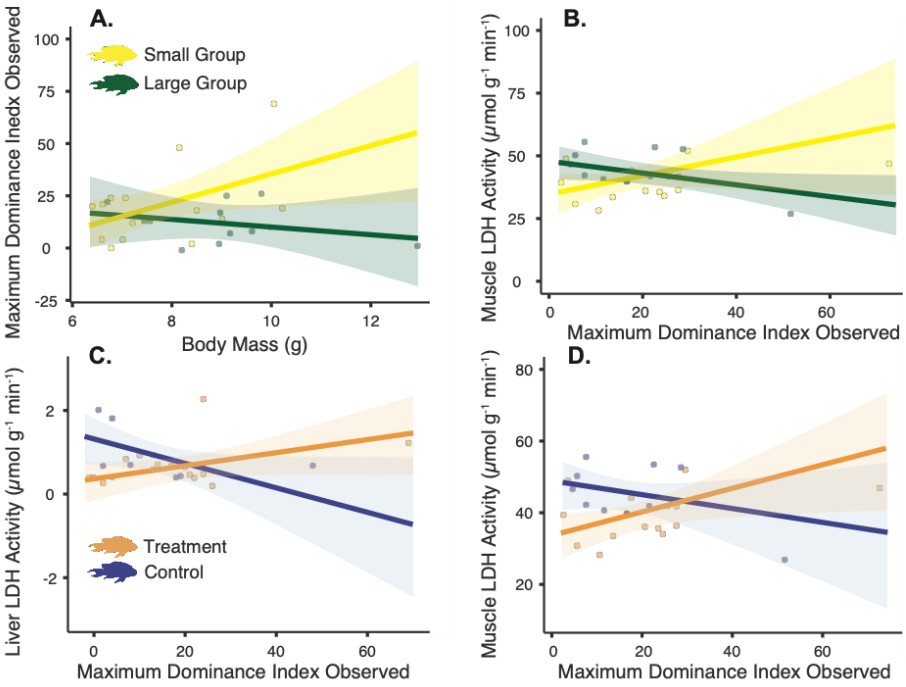

Figure 2. Relationships between the maximum dominance we observed (Maximum Dominance Index) and body mass (A), Muscle LDH activity (B), Liver LDH activity (C), and Muscle LDH activity (D). Small and large groups (A,B) are represented by yellow and green, respectively, whereas treatment and control groups (C,D) are represented by orange and blue, respectively. Enclosed circles represent observed scores. Note, the directionality and patterns of the relationship remain when we remove the two most extreme data points.

538 **File S1.** Post-hoc pairwise comparisons for dominance index GLMM.

539 **File S2.** Post-hoc pairwise comparisons for affiliation index GLMM.

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541 **Appendix 1.** Statistical parameters for the saturated GLMM for dominance indices.

	F	Num df	Den df	p
Treatment	5.4276	1	528	0.020
Perturbation	9.96e-4	1	528	0.975
Time Point	1.1063	3	528	0.346
Group Size	8.1205	1	528	0.005
Subject	142.4906	2	528	< .001
Treatment ⁕ Perturbation	0.3960	1	528	0.529
Treatment ⁕ Time Point	0.4287	3	528	0.733
Perturbation ⁕ Time Point	0.0933	3	528	0.964
Treatment ⁕ Group Size	4.7720	1	528	0.029
Perturbation ⁕ Group Size	3.23e-4	1	528	0.986
Time Point ⁕ Group Size	0.5007	3	528	0.682
Treatment ⁕ Subject	30.4299	2	528	< .001
Perturbation ⁕ Subject	2.5110	2	528	0.082
Time Point ⁕ Subject	3.8386	6	528	< .001
Group Size ⁕ Subject	4.3436	2	528	0.013
Treatment ⁕ Perturbation ⁕ Time Point	0.0426	3	528	0.988
Treatment ⁕ Perturbation ⁕ Group Size	0.3010	1	528	0.583
Treatment ⁕ Time Point ⁕ Group Size	0.5125	3	528	0.674
Perturbation ⁕ Time Point ⁕ Group Size	0.1396	3	528	0.936
Treatment ⁕ Perturbation ⁕ Subject	2.6552	2	528	0.071
Treatment ⁕ Time Point ⁕ Subject	3.7067	6	528	0.001
Perturbation ⁕ Time Point ⁕ Subject	0.8327	6	528	0.545
Treatment ⁕ Group Size ⁕ Subject	2.1819	2	528	0.114
Perturbation ⁕ Group Size ⁕ Subject	0.6660	2	528	0.514
Time Point ⁕ Group Size ⁕ Subject	4.9333	6	528	< .001
Treatment ⁕ Perturbation ⁕ Time Point ⁕ Group Size	0.0948	3	528	0.963
Treatment ⁕ Perturbation ⁕ Time Point ⁕ Subject	0.5351	6	528	0.782

	F	Num df	Den df	p
Treatment ✻ Perturbation ✻ Group Size ✻ Subject	1.9841	2	528	0.139
Treatment ✻ Time Point ✻ Group Size ✻ Subject	3.5868	6	528	0.002
Perturbation ✻ Time Point ✻ Group Size ✻ Subject	0.5734	6	528	0.752
Treatment ✻ Perturbation ✻ Time Point ✻ Group Size ✻ Subject	0.8689	6	528	0.517

Numerator degrees of freedom, Num df; Denominator degrees of freedom, Den df.

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546 **Appendix 2.** Statistical parameters for the saturated GLMM for Affiliation indices.

	F	Num df	Den df	p
Group Size	0.00442	1	22.0	0.948
Treatment	3.12960	1	22.0	0.091
Trial #	10.75636	1	506.0	0.001
Subject	6.41299	2	506.0	0.002
Hours	1.93144	3	506.0	0.124
Group Size ✻ Treatment	1.25136	1	22.0	0.275
Group Size ✻ Trial #	1.15353	1	506.0	0.283
Treatment ✻ Trial #	1.17067	1	506.0	0.280
Group Size ✻ Subject	0.49489	2	506.0	0.610
Treatment ✻ Subject	1.57884	2	506.0	0.207
Trial # ✻ Subject	0.83349	2	506.0	0.435
Group Size ✻ Hours	1.16357	3	506.0	0.323
Treatment ✻ Hours	1.71051	3	506.0	0.164
Trial # ✻ Hours	4.70137	3	506.0	0.003
Subject ✻ Hours	0.62838	6	506.0	0.708
Group Size ✻ Treatment ✻ Trial #	0.16617	1	506.0	0.684
Group Size ✻ Treatment ✻ Subject	2.01673	2	506.0	0.134
Group Size ✻ Trial # ✻ Subject	0.71484	2	506.0	0.490
Treatment ✻ Trial # ✻ Subject	0.98941	2	506.0	0.373
Group Size ✻ Treatment ✻ Hours	2.67528	3	506.0	0.047
Group Size ✻ Trial # ✻ Hours	1.04450	3	506.0	0.372
Treatment ✻ Trial # ✻ Hours	1.98722	3	506.0	0.115
Group Size ✻ Subject ✻ Hours	0.57562	6	506.0	0.750
Treatment ✻ Subject ✻ Hours	0.36084	6	506.0	0.904
Trial # ✻ Subject ✻ Hours	0.95883	6	506.0	0.453
Group Size ✻ Treatment ✻ Trial # ✻ Subject	0.59126	2	506.0	0.554

	F	Num df	Den df	p
Group Size ✻ Treatment ✻ Trial # ✻ Hours	0.26749	3	506.0	0.849
Group Size ✻ Treatment ✻ Subject ✻ Hours	0.49915	6	506.0	0.809
Group Size ✻ Trial # ✻ Subject ✻ Hours	0.28290	6	506.0	0.945
Treatment ✻ Trial # ✻ Subject ✻ Hours	0.52073	6	506.0	0.793
Group Size ✻ Treatment ✻ Trial # ✻ Subject ✻ Hours	0.42951	6	506.0	0.859

Numerator degrees of freedom, Num df; Denominator degrees of freedom, Den df.

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549 **Appendix 3.** Statistical parameters for saturated GLMs for female-level effects.

Morphometrics on Max Dom	**SS**	**df**	**F**	**p**
Model	2442.294	11	0.8988	0.564
BM	164.304	1	0.6651	0.428
Group Size	94.829	1	0.3839	0.545
CSI	15.386	1	0.0623	0.807
HSI	45.337	1	0.1835	0.675
Treatment	25.774	1	0.1043	0.751
Group Size ✻ BM	233.23	1	0.9442	0.348
Group Size ✻ Treatment	0.12	1	4.86E-04	0.983
Group Size ✻ CSI	75.255	1	0.3046	0.59
CSI ✻ Treatment	233.335	1	0.9446	0.348
HSI ✻ Treatment	524.486	1	2.1232	0.167
HSI ✻ Group Size	133.205	1	0.5392	0.475
Residuals	3458.322	14		
Liver LDH				
Model	3.67436	11	1.2794	0.338
Group Size	0.3807	1	1.4581	0.25
Treatment	0.20812	1	0.7971	0.39
Max Dominance	1.05367	1	4.0356	0.068
BM	0.00819	1	0.0314	0.862
Muscle LDH	0.04969	1	0.1903	0.67
Muscle CS	0.68879	1	2.6381	0.13
Liver CS	0.45984	1	1.7612	0.209
Group Size ✻ Treatment	0.05786	1	0.2216	0.646
Group Size ✻ Max Dominance	0.6402	1	2.452	0.143
Treatment ✻ Max Dominance	0.77553	1	2.9703	0.11
Group Size ✻ Treatment ✻ Max Dominance	0.29952	1	1.1472	0.305
Residuals	3.13309	12		
Muscle LDH				
Model	946.99	10	2.9452	0.036
BM	58.17	1	1.809	0.202
Treatment	113.65	1	3.5344	0.083
Max Dominance	11.27	1	0.3504	0.564
Group Size	23.26	1	0.7233	0.41
Muscle CS	175.42	1	5.4555	0.036
Liver CS	83.74	1	2.6042	0.131
Group Size ✻ Treatment	10.61	1	0.3301	0.575

Group Size ✻ Max Dominance	81.32	1	2.529	0.136
Treatment ✻ Max Dominance	82.1	1	2.5534	0.134
Group Size ✻ Treatment ✻ Max Dominance	2.57	1	0.0799	0.782
Residuals	418	13		
Liver CS				
Model	0.25927	11	1.023	0.481
Group Size	0.00982	1	0.426	0.526
Max Dominance	0.10208	1	4.432	0.057
Liver LDH	0.04057	1	1.761	0.209
Treatment	0.11943	1	5.185	0.042
BM	0.03294	1	1.43	0.255
Muscle LDH	0.03466	1	1.505	0.243
Muscle CS	0.04297	1	1.866	0.197
Group Size ✻ Treatment	0.00676	1	0.294	0.598
Group Size ✻ Max Dominance	0.13513	1	5.867	0.032
Treatment ✻ Max Dominance	0.00247	1	0.107	0.749
Group Size ✻ Treatment ✻ Max Dominance	0.02706	1	1.175	0.3
Residuals	0.27639	12		
Muscle CS				
Model	16.08533	11	1.33472	0.313
Group Size	0.02751	1	0.02511	0.877
Treatment	4.95256	1	4.52047	0.055
Liver LDH	2.8903	1	2.63813	0.13
Muscle LDH	3.07478	1	2.80651	0.12
Liver CS	2.04391	1	1.86558	0.197
BM	0.93274	1	0.85136	0.374
Max Dominance	3.31084	1	3.02198	0.108
Group Size ✻ Treatment	0.00161	1	0.00147	0.97
Group Size ✻ Max Dominance	3.34224	1	3.05064	0.106
Treatment ✻ Max Dominance	0.34866	1	0.31824	0.583
Group Size ✻ Treatment ✻ Max Dominance	1.75561	1	1.60244	0.23
Residuals	13.14703	12		

550 BM, body mass; Max Dominance, Maximum Dominance Index Observed; CSI, cardio somatic index; HIS, hepatic
551 somatic index.
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553 **Appendix 4.** Statistical parameters for final (minimal) GLM for female-level effects.

Liver LDH				
Model	0.841	5	0.507	0.767
BM	0.196	1	0.593	0.451
Treatment	0.118	1	0.356	0.558
Muscle LDH	0.263	1	0.794	0.385
Mean Activity	0.131	1	0.394	0.538
Treatment ✻ Mean Activity	0.173	1	0.522	0.479
Residuals	5.967	18		
Muscle LDH				
Model	286.556	6	0.71265	0.644
BM	37.637	1	0.5616	0.463
Treatment	105.763	1	1.57816	0.225
Group Size	7.757	1	0.11575	0.738
Mean Activity	46.458	1	0.69323	0.416
Treatment ✻ Mean Activity	0.127	1	0.00189	0.966
Group Size ✻ Mean Activity	27.164	1	0.40534	0.532
Residuals	1206.302	18		

554 BM, body mass; Max Dominance, Maximum Dominance Index Observed; Shading, $p < 0.05$.