Social rank and species-specific differences influence cooperation and conflict in anemonefish societies

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Many animal groups consist of dominance hierarchies. Theoretical models suggest that higher-ranked individuals should increase aggression but should decrease helping unless the cost of helping decreases with higher rank. Most empirical tests focus on systems with kin selection, whereas tests for groups in which individuals are unrelated are rare. Here we used two anemonefish species to test hypotheses of variation in helping and aggression with respect to social rank. We assessed behavioural frequencies for each rank in Amphiprion percula and A. perideraion groups and performed a removal experiment to determine whether behaviours were rank- or size-specific. Overall, we found A. perideraion performed more aggression and less helping than A. percula, possibly due to a difference in ecological constraints. In both species, we found consistent differences in behaviour between ranks, with higher ranks performing both aggression and helping more often. Despite low relatedness within groups, we found that lower-ranked individuals helped in both species. When we experimentally promoted individuals and controlled for group size, their behaviour became indistinguishable from that of individuals established in the higher rank. Thus, for the first time, we quantified helping for nonbreeding individuals in a marine fish, showed that behavioural patterns are rank-specific, and confirmed that individuals adjust both aggression and helping to their likelihood of inheriting a breeding position.

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In many animal societies, group members are organized into dominance hierarchies or queuing systems, based for example on size, fighting ability or age (Buston, 2003a; Cant, 2000; Shreeves & Field, 2002). Within these hierarchies, cooperative effort, that is, helping that is beneficial to the group or territory, often varies dramatically between individuals (Balshine et al., 2001; Clutton-Brock et al., 2008; Siiter et al., 2006; Wong, 2011). Several theoretical frameworks exist that make predictions about rank-specific behaviours. In most cases theory suggests that individuals should reduce the amount of helping and increase the amount of aggression they perform when they are of higher rank and in larger groups (Cant & Field, 2001, 2005; Cant, Llop, et al., 2006; Field et al., 2006). Higher-ranked subordinates have a higher probability of future fitness returns from inheriting the dominant position and should therefore put less effort into helping (Cant & Field, 2001, 2005; Field et al., 2006) and more effort into aggression defending their rank and testing dominants (Cant, Llop, et al., 2006). However, more aggression and more helping would be expected for higher ranks when the cost of helping is lower in higher ranks (Cant & Field, 2005). Many models primarily consider kin selection benefits from helping related group members (Hamilton, 1964; Queller, 1994; West-Eberhard, 1975), but even when the degree of relatedness is very low, helping by lower ranks can still be predicted if group members gain future fitness benefits from inheriting territories and dominant status (Kingma, 2017; Kokko & Johnstone, 1999; Kokko et al., 2001, Woolfenden & Fitzpatrick, 1978), or if they have to ‘pay-to-stay’ (Balshine-Earn et al., 1998). Empirical tests of these theoretical predictions have so far been conducted mainly in insect and mammalian groups (e.g. Amsalem & Hefetz, 2011; Cant, English, et al., 2006; Cronin & Field, 2007; Jandt et al., 2014; Thavarajah et al., 2014).

Even though some fish societies bear striking similarities in social organization to mammalian and insect groups, marine fishes in particular display a number of key differences to the typical sociality of terrestrial taxa (Rueger et al., 2021). First, most social marine fish species reside in unrelated groups (Buston et al., 2007;...
Salles et al., 2016). This difference is important because the lack of indirect benefits from group living will greatly alter the payoffs to subordinates of peacefully accepting nonbreeding status, and hence could change the expression of underlying and overt conflict. Second, subordinates do not provide active helping in the form of alloparental care, which is exhibited in terrestrial taxa even by unrelated subordinates (Dunn et al., 1995; Queller et al., 2000). The lack of active provision of help to dominants would alter the payoffs to dominants of accepting and tolerating subordinates within groups, and hence change the expression of underlying and overt conflict. Even so, subordinate marine fishes likely express other behaviours that are beneficial to dominants, such as defence against predators, territory maintenance and cleaning (Fricke, 1979; Iwata & Manbo, 2013; Ross, 1978), although these behaviours have rarely been quantified or studied for specific ranks. The fact that marine fishes experience different payoffs from social group living compared to most terrestrial taxa means that they can yield novel insights into the dynamics of dominance hierarchies and can advance our understanding of sociality in animals more broadly.

Here we used anemonefishes (genus Amphiprion) to examine differences in helping and aggression between ranks and to experimentally test whether these differences are likely to be rank-specific, i.e. whether there are predictable differences in behaviour with respect to rank as well as changes in behavioural patterns as an individual changes rank. Anemonefishes form symbiotic relationships with their host anemone (Fautin, 1992). They are sequential hermaphrodites and form strict size hierarchies within groups of unrelated individuals, where groups typically consist of one dominant breeding pair and zero to five unrelated subordinates (Buston, 2004a; Buston & Cant, 2006; Fautin, 1992; Fricke, 1979). The female is always the largest individual (rank 1), the male is the second largest (rank 2) and all other fish are nonbreeders that get progressively smaller down the hierarchy (Buston, 2003a; Fricke & Fricke, 1977). Rank ascension only happens when a higher rank disappears (Buston, 2003a, 2004b). Although subordinate anemonefishes are not known to help dominants rear offspring (but see Phillips et al., 2020), they do cooperate by manipulating their growth rate to stay smaller than the individuals higher up in the hierarchy (Buston, 2003a). Also, alternative metrics of helping, namely territory defence (chasing predators away from the group; Fricke, 1979; Iwata & Manbo, 2013; Ross, 1978) and territory maintenance (cleaning the anemone; Mariscal, 1966) can be observed and quantified. Although group members are generally unrelated (Buston et al., 2007), we might still expect nonbreeders to help because they stand to eventually inherit the breeding territory (Buston, 2004a) and there is potential for synergistic effects between larger groups and the health and size of the anemone host (Holbrook & Schmitt, 2005). However, we might expect the cost of helping to be higher for lower ranks in anemonefishes, since the hierarchy is based on size and any activity might increase exposure to predators or be differentially energetically costly (Buston, 2003b; Branconi et al., 2020).

Here we focused on two closely related anemonefish species common in the Indo-Pacific; Amphiprion percula and A. perideraion. The two species both primarily inhabit the same anemone species, Heteractis magnifica, and are otherwise ecologically similar (Elliott & Mariscal, 2001). However, while A. percula do not leave their host anemone after settlement (Branconi et al., 2020), large, dominant A. perideraion have been observed moving to a different anemone occupied by A. percula and usurping them (Rueger et al., 2018). If individuals in A. perideraion are capable of moving, we hypothesized that individuals have other options than waiting to inherit breeding status (Mitchell & Dill, 2005), which is a key assumption of the theory. As a result, rank-related variation in social behaviours may be diluted due to their increased capacity for movement. Therefore, examining theoretical expectations using both species as counterparts serves to provide a more rigorous test of the hypothesis.

To investigate whether and how helping and aggression vary between social ranks and species, we used a combination of field observations and experimental techniques to test the following specific predictions in A. percula and A. perideraion. (1) Rank-specific predictions: the frequency of aggression will increase with rank but the frequency of helping will decrease, in line with expectations for animals that live in similar societies (Cant, Llop, et al., 2006; Field et al., 2006). Alternatively, if the cost of helping decreases with increased rank, the frequency of both aggression and helping will increase with higher rank (Cant & Field, 2005). (2) Species-specific predictions: the frequency of aggression and helping will be similar given the species are ecologically similar. Alternatively, if ecological constraints are indeed relaxed in A. perideraion, group stability may be lower, due to higher propensity of movement between groups, and helping may be less frequent in this species (Kingma, 2017).

METHODS

The study was conducted in Kimbe Bay, Papua New Guinea, in September–October 2018 and May 2019 (totalling 3 months of field work). All observations and experimental manipulations were carried out in situ on wild anemonefish groups. We used 16 A. percula groups and 12 A. perideraion groups, all associated with H. magnifica hosts. For A. percula, groups comprised four (N = 12) or five fish (N = 4). For A. perideraion, groups comprised four fish (N = 9) or five fish (N = 3). Ten A. percula groups and three A. perideraion groups were observed breeding during the experiment. To ensure that groups used in the study were independent, we recorded their GPS coordinates and used an underwater tagging system to identify specific groups. Each group was marked with a numbered identification tag attached to the substrate next to the anemone, which was removed only at the completion of the study.

Behavioural Differences

Unmanipulated groups were filmed without divers present with a GoPro Hero5 for 12 min from a 1.5–2.5 m distance. This filming duration is consistent with other studies that have recorded video footage of anemonefish behaviours in the wild (Wong et al., 2017; Trnski et al., 2020) and was appropriate given the logistics involved with data collection at depth. For this component of the study, each group was filmed once only. Behaviours were scored from videos using the BORIS program (Friard & Gamba, 2016) and following an ethogram based on (Wong et al. 2013; Appendix Table A1). For each video, 10 of the 12 min of footage was watched with 1 min at the start and end of each video being discarded to account for diver disturbance during camera set-up and removal. To account for differences in the time each fish was visible in the frame, time out of sight was noted and the frequencies of all behaviours were analysed per minute of observation. Every individual in the group was scored separately and continuously in one sequence to make behaviour scores as accurate as possible, meaning that 10 mins of continuous footage was watched per individual in the group. Individuals were recognized in the video footage by their unique markings and visually discernible size differences that arise due to the structured size hierarchy within groups (Buston, 2003b; Nelson et al., 1994). Behaviours fell into four broad categories: (1) aggressive, (2) submissive, (3) neutral, (4) helping (Appendix Table A2). For all interactive behaviours, the individual receiving the submission or aggression was recorded. Behaviours were scored for
ranks 1 to 5. The analysis below focuses on aggression and helping, but some analysis for submissive and neutral behaviours can be found in the Appendix.

### Rank Ascension Experiment

To examine variability in helping and aggression between ranks, we removed the rank 3 individual from half of the groups of *A. percula* (*N* = 8) and *A. perideraion* (*N* = 6) (i.e. treatment groups; Fig. 1). From the other half of the groups (i.e. control groups; Fig. 1) we removed the lowest-ranking individual (rank 4 or 5 depending on the group size). This was done to control for the reduced number of group members and to enable comparison of rank 4 behaviours from the treatment (individual ascending to rank 3) with rank 3 behaviours from the control groups. Removed individuals were held in aerated sea water tanks, provided with shelter and fed twice daily with freeze-dried brine shrimp (Omega One) and fish pellets (New Life Spectrum). Fish were replaced into their original groups at the end of the study, 2 days after their removal. They initially received aggressive displays and attacks, but all were eventually accepted back into their groups. Groups were filmed as above at four time points: (1) immediately prior to removal of the rank 3 (or lowest rank in control groups), (2) immediately after removal of the fish, (3) 24 h after removal and (4) 48 h after removal. After the last filming session, all fish in the group were caught using hand nets and their standard length (SL) measured to the nearest 0.5 mm using callipers. To account for size differences that might influence behaviours, we calculated the size ratio of the focal individual and the individual immediately above it in rank (focal individual SL: individual above in the hierarchy SL). The mean size ratio between all group members was higher in *A. perideraion* (mean ± SE = 0.752 ± 0.014) than in *A. percula* (mean ± SE = 0.713 ± 0.011), meaning fish were closer together in size in *A. perideraion*. This effect was even stronger when just considering subordinate individuals (ranks 3–5; *A. perideraion*, mean ± SE = 0.765 ± 0.019; *A. percula*, mean ± SE = 0.697 ± 0.015). To account for any ecological effects of anemone size, we measured the long and short diameter of the anemone’s tentacle crown. Anemone size was then

![Figure 1](image_url)

**Figure 1.** Experimental design of the rank ascension experiment conducted with groups of *A. percula* (*N* = 16) and *A. perideraion* (*N* = 12) in Kimbe Bay, Papua New Guinea. In treatment groups, the rank 3 (R3) was removed allowing for rank ascension by rank 4 (R4). In control groups, the lowest-ranked group member was removed to control for group size reduction. TP = Time point. R = rank.
calculated as the area of an ellipse (\(\pi \times \text{major axis}/2 \times \text{minor axis}/2\)). Anemones hosting \(A.\ percula\) groups were smaller on average (mean ± SE = 383 ± 26 cm\(^2\)) than those with \(A.\ perideraion\) groups (mean ± SE = 676 ± 55 cm\(^2\)).

Data Analysis

We used R (R Core Team, 2020) and the ‘lme4’ package (Bates et al., 2015) for linear mixed model analysis (LMM). We used the Akaike information criterion (AIC; Sakamoto et al., 1986) for model selection. If \(\Delta\text{AIC}\) was below 2, likelihood ratio tests identified the best-fit model, with preference for the simpler model. Significance tests for LMMs were performed by likelihood ratio tests of the full model with the effect in question against the model without the effect. No obvious deviations from homoscedasticity were detected by visually inspecting the residual plots. No outliers or high variance inflation factors (VIF) were detected in any of the best-fit models, using the ‘performance’ package (Lüdecke et al., 2020).

Behavioural differences

To test the differences between ranks and species, we fitted separate LMMs for the two behavioural categories: aggression and helping. For helping/min we excluded parental behaviours (see Appendix Table A1 for definition), since these were only recorded in \(A.\ percula\) during this component of the study. Fixed factors included fish species (\(A.\ percula, A.\ perideraion\)) and individual rank (ranks 1–5). Covariates included number of fish in each group (group size), anemone size and mean size ratio per group. Mean size ratio was used rather than individual size ratios because this allowed all ranks to be included in the model (rank 1 has no individual above it in rank to compare to, so could not have an individual size ratio calculation). Model selection was performed as above. Group ID was used as a random variable to

Figure 2. Behavioural frequencies (behaviours/min) for each rank (rank 1, \(N = 28\); rank 2, \(N = 28\); rank 3, \(N = 28\); rank 4, \(N = 28\); rank 5, \(N = 7\)) in the size hierarchy in the anemonefishes \(A.\ percula\) and \(A.\ perideraion\). (a) Aggression; (b) helping. Central bar: median; boxes: lower and upper quartiles; whiskers: ±1.5 × interquartile range; dots: outliers.
account for nonindependence of individual behaviours within a group.

**Rank ascension experiment**

To test differences in behaviour between the treatment and control groups, separate LMMs were fitted for each species (*A. percula*, *A. perideraion*). Only rank 3 from the control groups and rank 4 from the treatment groups were tested. The two behavioural categories of primary interest were used as response variables (aggression/min, helping/min). Fixed factors were time (categorical: premanipulation, 0 h postmanipulation, 24 h postmanipulation, 48 h postmanipulation), and treatment (categorical: treatment, control) and their interaction. To account for differences in relative size, we included size ratio between the focal individual and the individual immediately above it in rank (continuous). Group ID was used as a random variable to account for repeated measures of the same individuals. We compared the behaviour of the rank 3 in control groups (lowest rank removed) with the behaviour of the ascended rank 4 in treatment groups (rank 3 removed) using post hoc tests on the difference in behaviours between time points using the ‘emmeans’ package (Lenth, 2020). We accounted for multiple testing using multivariate t distribution adjustments.

**Ethical Note**

This study was approved by the University of Wollongong Animal Ethics Committee, permit number AE18/06.

**RESULTS**

**Behavioural Differences**

**Aggression**

There were significant differences in the frequency of aggression between ranks ($\chi^2 = 7.462, P < 0.001$) and species ($\chi^2 = 7.433, P = 0.006$; Fig. 2a). The fixed effects explained 42% of the variance ($R^2 = 0.583, R^2_m = 0.417$). The best-fit model included group size ($\chi^2 = 4.786, P = 0.029$), but not anemone size, size ratio or interactions between any fixed effects. The model estimates show that for each species, ranks 1–3 were more aggressive than the lower ranks (Appendix Table A3), and, on average, rank 4 showed 48% ($\pm 7\%$ SE) less aggression than rank 1. Overall, aggression was

![Figure 3](image-url). Aggressive behaviours (number/min) recorded for each rank (1–4) in the anemonefishes *A. percula* (*N* = 16) and *A. perideraion* (*N* = 12). Target: the individual the aggression was directed towards (rank 1–5). Central bar: median; boxes: lower and upper quartiles; whiskers: ±1.5 x interquartile range.
23% (± 8.5% SE) higher in A. perideraion compared to A. percula (Appendix Fig. A1). All aggression was directed down the hierarchy towards smaller, lower-ranked group members (Fig. 3).

Helping
There were significant differences in the frequency of helping between ranks ($\chi^2 = 52.567, P < 0.001$) and species ($\chi^2 = 4.289, P = 0.038$; Fig. 2b). The best-fit model included size ratio ($\chi^2 = 1.037, P = 0.308$), but not group size, anemone size or any interactions. The fixed effects explained 31% of the variance ($R^2_f = 0.496, R^2_{mp} = 0.308$). Helping was predicted at a 22% (± 11% SE) higher rate for A. percula compared to A. perideraion. For each species, ranks 1–3 helped more than the lower ranks and on average rank 4 showed 51% (± 9% SE) less helping than rank 1 (Appendix Table A4).

The specific helping behaviours performed also differed between ranks (Fig. 4). For example, in A. percula, interaction with the anemone ('cleaning') made up approximately 39% of helping for ranks 1 and 2, whereas for ranks 4 and 5 this made up most of the helping (53% and 75%, respectively; Fig. 4).

Rank Ascension Experiment

Aggression
There was a significant interaction between treatment (treatment/control) and time (premanipulation/postmanipulation) for the frequency of aggression in both A. percula ($\chi^2 = 12.252, P = 0.007$) and A. perideraion ($\chi^2 = 14.113, P = 0.003$). When group size was reduced (postmanipulation) aggression declined steeply in rank 3 controls, whereas it did not change in rank 4 treatments (Fig. 5a, b). There was a significant effect of size ratio in A. percula (log estimate (± SE) = 0.460 ± 0.532, $\chi^2 = 56.211, P < 0.001$), with individuals closer in size being more aggressive, but not in A. perideraion (log estimate (± SE) = 0.078 ± 0.783, $\chi^2 = 0.019, P = 0.888$).

Holding size ratio constant, post hoc comparisons between premanipulation and 48 h postmanipulation showed an average decline in aggression for rank 3 controls of 33% in A. percula (log estimate = −0.331, 95% confidence intervals = −0.058, −0.604) and a decline of 69% for A. perideraion (log estimate = −0.699, confidence intervals = −1.086, −0.313). In contrast, rank 4 individuals did not change significantly, with the frequency of aggression slightly increasing in A. percula (log estimate = 0.189, confidence intervals = −0.278, 0.657) and A. perideraion (log estimate = −0.002, confidence intervals = −0.639, 0.008).

Helping
There was a significant interaction between treatment (treatment/control) and time (premanipulation/postmanipulation) for the frequency of helping in A. perideraion ($\chi^2 = 14.113, P = 0.003$) but not in A. percula ($\chi^2 = 0.962, P = 0.811$; Fig. 5c, d). Size ratio was not significant (A. percula, log estimate (± SE) = 1.062 ± 0.666, $\chi^2 = 2.711, P = 0.099$; A. perideraion, log estimate (± SE) = 0.475 ± 0.469, $\chi^2 = 1.210, P = 0.271$).

In A. percula, helping in rank 4 was highest 48 h after removal of rank 3, when helping was performed on average at a 2.8 times higher rate compared to the premanipulation time point (mean helping/min ± SE, time point 1: 0.35 ± 0.1; time point 4: 0.96 ± 0.3). In A. perideraion, helping in rank 4 in the treatment was highest 24 h after removal of rank 3, when helping was performed on average at a 1.8 times higher rate compared to the premanipulation time point (mean helping/min ± SE, time point 1: 0.21 ± 0.05; time point 3: 0.37 ± 0.16).

DISCUSSION
We have shown that in two anemonefish species, nonbreeders in groups of unrelated individuals display helping that is potentially beneficial to the group. We found differences in levels of aggression and helping among ranks in the size hierarchies, with consistently
higher frequencies of helping as well as aggression in higher ranks (larger, more dominant individuals) in both species. While these rank-specific patterns were the same among *A. percula* and *A. perideraion*, species differed in overall levels of aggression and helping, with the species where ecological constraints seem to be relaxed (*A. perideraion*) showing more aggression and less helping overall.

**Quantification of Helping**

Our findings contribute to the growing body of literature disentangling the causes of sociality in groups of unrelated vertebrates. For anemonefishes, the reasons for subordinates to stay in groups of nonrelatives and forego reproduction have been found to range from future fitness benefits through territory inheritance (Buston, 2004a) to the presence of social constraints (Buston, 2003b; Rueger et al., 2018) and environmental constraints (Branconi et al., 2020). However, why dominants tolerate unrelated, nonbreeding subordinates has remained unclear, since subordinates have not been shown to directly increase dominants' reproductive output, at least in the short term (Buston, 2004b). Here, however, we provide evidence to show that subordinates do help and hence a reason why breeders tolerate them. These behaviours, namely the cleaning and massaging of the anemone and deterring anemone predators, are likely to be cooperative as they entail short-term fitness costs (time and energy that could be focused on other activities such as feeding) and yet provide indirect benefits to dominants. From the subordinate's perspective, there is minimal relatedness among group members (Buston et al., 2007) and therefore no inclusive fitness to be gained through helping. Rather, helping effort is likely to be beneficial as it increases the quality of the territory (anemone), which in turn increases survival, access to resources and later inheritance of a higher-quality territory upon death of the dominants (Kingma et al., 2014; Kokko et al., 2001). Since a substantial proportion of the helping observed in this study involved interacting with the anemone, it is likely that enhancement of territory quality is a key benefit of helping, whereby future benefits are generated for both the dominants and helpful subordinates.

**Rank Comparison**

Since the dominance hierarchy in anemonefishes is organized by size (Buston, 2003b), we suggest that lower ranks may not be able to bear the cost of helping, as has been found in some birds, mammals and other fishes (Boland et al., 1997; Clutton-Brock et al.,

![Figure 5](image-url)
Our results confirm predictions that lower ranks should perform less helping if the cost of helping is greater for lower ranks (Cant & Field, 2005). Taborsky (1984) demonstrated the cost of helping in a cooperatively breeding cichlid, by showing that subordinates performing helping behaviours grew slower than those that did not help. In our study, lower ranks spent more time performing submissive behaviours and the lowest ranks did not perform many of the behaviours observed in the higher ranks. Instead, they were seemingly occupied with feeding and avoiding other group members (T. Rueger, personal observation), presumably to avoid eviction (Buston, 2003b; Rueger et al., 2018). In our experiment, lower ranks slightly increased helping when prompted in rank, but this effect was only significant in one species. This is likely due to the short timeframe of our experiment which did not allow rank 4 individuals ascending in rank to increase their size, and thus the cost of helping remained high. Further, both our observations and experimental results confirm predictions about increased aggression with higher rank (Cant; Llop et al., 2006) and are in line with findings for other fishes organized in dominance hierarchies (Collette & Brown, 2011; Dey et al., 2013). Thus, our results show the importance of studying aggression and helping simultaneously and for each individual rank, and the importance of extending empirical testing to unusual model taxa.

Species Comparison

Interestingly, when comparing two species that are closely related and share a very similar ecology, we found some distinct differences in the degree to which specific behaviours were displayed. The causes of higher aggression in A. perideraion compared to A. percula are not known but may be partially related to smaller size differences between the lower ranks in A. perideraion compared to A. percula. Size ratios in fish groups are known to vary with respect to position in the hierarchy (Buston & Cant, 2006; Wong, 2011). When lower ranks are closer in size, as is observed in A. perideraion, individuals may need to be more aggressive to protect their rank from those adjacent to them in the hierarchy. Additionally, A. perideraion exhibited lower levels of helping (territory maintenance and defence) than A. percula. The difference is likely to be due to relaxed ecological constraints in A. perideraion, although future experiments are needed to confirm this. A higher rate of movement between groups may lead to less group stability and this in turn could lower the prospects of territory inheritance. In a society with low relatedness, where helping actions are primarily self-serving through increasing the quality of the territory that will be inherited, lower prospects of inheritance are predicted to lead to less helping, as has been found in many bird species (Kingma, 2017). Future research should focus on the nuanced differences between species and experimentally explore the ecological factors that may lead closely related species of anemonefishes to pursue different social strategies and show different behavioural frequencies.

Conclusion

Our study has shown strong support for some theoretical predictions pertaining to variation in aggression and helping levels between ranks in animal groups with dominance hierarchies, providing the first empirical tests for these models in marine fishes. Another important aspect of this study was the quantification of behaviours of subordinates that are helpful to the group and/or territory. Although we did not observe alloparental brood care, we did find significant levels of helping in subordinates, which is likely to have long-term impacts on the fitness of dominant breeders via effects on the anemone host. This contributes to the growing evidence for how sociality has evolved and persists when group members are unrelated. Finally, we have shown that there are interspecific differences in aggression and helping between closely related species. These differences warrant further investigation using broader comparative approaches in a wider range of species and likely represent the next frontier in sociality research.

Author Contributions

T.R., S.J.H. and M.Y.W. designed the research, T.R. and S.J.H. performed fieldwork, T.R. performed video and data analysis, T.R., S.J.H. and M.Y.W. wrote the manuscript.

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References

Amsalem, E., & Heftz, A. (2011). The effect of group size on the interplay between dominance and reproduction in Bombus terrestris. PLoS One, 6, Article e18238.
Balshine-Earn, S., Neat, F. C., Reid, H., & Taborsky, M. (1998). Paying to stay or paying to breed? Field evidence for direct benefits of helping behavior in a cooperatively breeding fish. Behavioral Ecology, 9, 432–438.
Balshine, S., Leach, B., Neat, F. Reid, H., Taborsky, M., & Werner, N. (2001). Correlates of group size in a cooperatively breeding cichlid fish (Neolamprologus pulcher). Behavioral Ecology and Sociobiology, 50, 134–140.
Bates, D., Maechler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. Journal of Statistical Software, 67, 1–48.
Boland, C. R. J., Heinsohn, R., & Cockburn, A. (1997). Experimental manipulation of brood reduction and parental care in cooperatively breeding white-winged choughs. Journal of Animal Ecology, 66, 683–691.
Branconi, R., Barbasch, T. A., Francis, R. K., Srinivasan, M., Jones, G. P., & Buston, P. M. (2020). Ecological and social constraints combine to promote evolution of non-breeding strategies in clownfish. Communications Biology, 3, 1–7.
Buston, P. M. (2003a). Social hierarchies: Size and growth modification in clownfish. Nature, 424, 145–146.
Buston, P. M. (2003b). Forcible eviction and prevention of recruitment in the clown anemonefish. Behavioral Ecology, 14, 576–582.
Buston, P. M. (2004a). Territory inheritance in clownfish. Proceedings of the Royal Society B: Biological Sciences, 271, 5252–5254.
Buston, P. M. (2004b). Does the presence of non-breeders enhance the fitness of breeders? An experimental analysis in the clown anemonefish Amphiprion percula. Behavioral Ecology and Sociobiology, 57, 23–31.
Buston, P. M., Bogdanowicz, S. M., Wong, A., & Harrison, R. G. (2007). Are clownfish groups composed of close relatives? An analysis of microsatellite DNA variation in Amphiprion percula. Molecular Ecology, 16, 3671–3678.
Buston, P. M., & Cant, M. A. (2006). A new perspective on size hierarchies in nature: Patterns, causes, and consequences. Oecologia, 149, 362–372.
Cant, M. A. (2000). Social control of reproduction in banded mangoodes. Animal Behaviour, 59, 147–158.
Cant, M. A., English, S., Reeve, H. K., & Field, J. (2006b). Escalated conflict in a social hierarchy. Proceedings of the Royal Society B: Biological Sciences, 273, 2977–2984.
Cant, M. A., & Field, J. (2001). Helping effort and future fitness in cooperative animal societies. Proceedings of the Royal Society B: Biological Sciences, 268, 1595–1594.
Cant, M. A., & Field, J. (2005). Helping effort in a dominance hierarchy. Behavioral Ecology, 16, 708–715.
Cant, M. A., Llop, J. B., & Field, J. (2006a). Individual variation in social aggression and the probability of inheritance: Theory and a field test. American Naturalist, 167, 837–852.
Clutton-Brock, T. H., Brotherton, P. N. M., O’Reain, M. J., & Griffin, A. S. (2000). Individual contributions to babysitting in a cooperative mongoose Suricata suricatta. Proceedings of the Royal Society B: Biological Sciences, 267, 301–305.
Clutton-Brock, T. H., Hodge, S. J., & Flower, T. P. (2008). Group size and the suppression of subordinate reproduction in Kalahari meerkats. Animal Behaviour, 76, 689–700.
**Table A1**

| Ethogram          | Behaviour                          | Description                                                                 |
|-------------------|------------------------------------|-----------------------------------------------------------------------------|
| **Aggressive**    | Chases                             | Fish rapidly swims towards conspecific and conspecific fish                  |
|                   | Bites                              | Fish bites another fish                                                    |
|                   | Jolts                              | Rapidly darts and turns prior to or during aggressive contact               |
|                   | Aggressive display                 | Fish meets another, turns side on with erect fins, stiffened body posture |
|                   | Flee                               | Fish rapidly swims away after being chased or bitten                        |
|                   | Body shake                         | Fish shakes body side to side in jolly fashion                             |
| **Submissive**    | Body shake                         | Fish shakes body side to side in jolly fashion                             |
|                   | Bite anemone                       | Fish bites mouth or tentacles of anemone                                   |
|                   | Massage anemone                    | Fish moves vigorously against anemone foot (often observed when anemone is contracting) |
|                   | Cleaning                           | Fish picks up sand or debris and spits it out outside anemone               |
|                   | Defense against predator           | Quick swims or bites towards heterospecifics, either potential egg predators (e.g. wrasses) or anemone predators (e.g. butterflyfishes) |
|                   | Defense against competitor         | Quick swims or bites towards heterospecific food competitors (e.g. damselfishes) |
|                   | Parental                           | Fish is within one body length of the nest. May be fanning or mouthing eggs |
| **Neutral**       | Meeting                            | Fish simultaneously approach each other to within 1 body length (may be followed by soft touch) |
|                   | Soft touch                         | Fish make contact with fins or body before separating again, preceded by meeting |
|                   | Follows                            | Fish follows another fish within 1 body length for at least a few seconds Fish cannot be seen because it enters anemone, moves under or behind anemone, or swims beyond the video frame. Measured in seconds |
| **Out of sight**  |                                    |                                                                            |

Adapted from Wong et al. (2013).

**Table A2**

| Broad behavioural categories used in the analysis for *A. percula* and *A. perideraion* |
|----------------------------------------|----------------------------------------------------------------|
| Behavioural category                  | Description                                                                 |
| Aggression                            | All aggression among group members, e.g. chases, bites                  |
| Submission                            | All submission among group members, e.g. body shake                     |
| Neutral                               | When group members were within 1 body length of each other but did not display aggression or submission |
| Helping                               | Behaviours beneficial to the group (e.g. aggression towards egg predators, aggression towards food competitors, parental care) or beneficial to the anemone (e.g. anemone cleaning/manipulating, aggression towards anemone predators) |

For a more detailed description of behaviours see Table A1.

**Table A3**

| Contrast                        | Estimate | SE   | df   | t     | p     |
|---------------------------------|----------|------|------|-------|-------|
| Rank 2 percula—Rank 1 percula   | 0.07439  | 0.071| 108  | 1.048 | 0.988 |
| Rank 3 percula—Rank 1 percula   | −0.00086 | 0.071| 108  | −0.012| 1     |
| Rank 3 percula—Rank 2 percula   | −0.07525 | 0.071| 108  | −1.06 | 0.988 |
| Rank 4 percula—Rank 1 percula   | −0.4764  | 0.071| 108  | −6.709| <0.0001 |
| Rank 4 percula—Rank 2 percula   | −0.55079 | 0.071| 108  | −7.756| <0.0001 |
| Rank 4 percula—Rank 3 percula   | −0.47546 | 0.071| 108  | −6.696| <0.0001 |
| Rank 5 percula—Rank 1 percula   | −0.60266 | 0.1105| 116 | −5.452| <0.0001 |
| Rank 5 percula—Rank 2 percula   | −0.67705 | 0.1105| 116 | −6.125| <0.0001 |
| Rank 5 percula—Rank 3 percula   | −0.6018  | 0.1105| 116 | −5.444| <0.0001 |
| Rank 5 percula—Rank 4 percula   | −0.12626 | 0.1105| 116 | −1.142| 0.579 |
| Rank 1 per—Rank 1 percula       | 0.23341  | 0.0849| 31.1 | 2.748 | 0.199 |
| Rank 1 per—Rank 2 percula       | 0.15902  | 0.1107| 78.6 | 1.436 | 0.912 |
| Rank 1 per—Rank 3 percula       | 0.23427  | 0.1107| 78.6 | 2.116 | 0.522 |
| Rank 1 per—Rank 4 percula       | 0.70981  | 0.1107| 78.6 | 6.411 | <0.0001 |
| Rank 1 per—Rank 5 percula       | 0.83607  | 0.1372| 115.9| 6.056 | <0.0001 |
| Rank 2 per—Rank 1 percula       | 0.3078   | 0.1107| 78.6 | 2.78  | 0.162 |
| Rank 2 per—Rank 2 percula       | 0.23341  | 0.0849| 31.1 | 2.748 | 0.199 |
| Rank 2 per—Rank 3 percula       | 0.30866  | 0.1107| 78.6 | 2.788 | 0.159 |
| Rank 2 per—Rank 4 percula       | 0.7842   | 0.1107| 78.6 | 7.083 | <0.0001 |
| Rank 2 per—Rank 5 percula       | 0.91046  | 0.1372| 115.9| 6.638 | <0.0001 |
| Rank 2 per—Rank 1 per           | 0.07439  | 0.0711 | 108 | 1.048 | 0.988 |
| Rank 3 per—Rank 1 percula       | 0.23255  | 0.1107| 78.6 | 2.1  | 0.533 |
| Rank 3 per—Rank 2 percula       | 0.15816  | 0.1107| 78.6 | 1.428 | 0.915 |
| Rank 3 per—Rank 3 percula       | 0.23341  | 0.0849| 31.1 | 2.748 | 0.199 |
| Rank 3 per—Rank 4 percula       | 0.70895  | 0.1107| 78.6 | 6.403 | <0.0001 |
| Rank 3 per—Rank 5 percula       | 0.83521  | 0.1372| 115.9| 6.09  | <0.0001 |
Species: ‘percula’ – *A. percula*; ‘peri’ – *A. perideraion*. Response variable: log aggression/min; fixed variables: rank, species, group size; random variable: group ID. Estimated using the ‘emmeans’ package in R.

### Table A4

Helping behaviours: model estimates for contrast between ranks 1–5 and species

| Contrast                  | Estimate  | SE    | df  | t     | P     |
|---------------------------|-----------|-------|-----|-------|-------|
| Rank 1 percula – Rank 2 percula | −0.06117  | 0.0901| 108 | −0.682| 1     |
| Rank 1 percula – Rank 3 percula | 0.19832   | 0.0901| 108 | 2.202 | 0.462 |
| Rank 1 percula – Rank 4 percula | 0.51342   | 0.0901| 108 | 5.701 | <0.0001|
| Rank 1 percula – Rank 5 percula | 0.67373   | 0.1395| 118.5| 4.831 | 0.0002|
| Rank 1 percula – Rank 1 peri | 0.26534   | 0.1009| 31.8 | 2.629 | 0.246 |
| Rank 1 percula – Rank 2 peri | 0.20397   | 0.1353| 86.6 | 1.508 | 0.886 |
| Rank 1 percula – Rank 3 peri | 0.46366   | 0.1353| 86.6 | 3.428 | 0.039 |
| Rank 1 percula – Rank 4 peri | 0.77876   | 0.1353| 86.6 | 5.757 | <0.0001|
| Rank 1 percula – Rank 5 peri | 0.93907   | 0.1724| 124.3| 5.446 | <0.0001|
| Rank 2 percula – Rank 3 percula | 0.2597    | 0.0901| 108 | 2.884 | 0.123 |
| Rank 2 percula – Rank 4 percula | 0.57497   | 0.0901| 108 | 6.383 | <0.0001|
| Rank 2 percula – Rank 5 percula | 0.73511   | 0.1395| 118.5| 5.271 | <0.0001|
| Rank 2 percula – Rank 1 peri | 0.32671   | 0.1333| 86.6 | 2.415 | 0.33  |
| Rank 2 percula – Rank 2 peri | 0.26534   | 0.1009| 31.8 | 2.629 | 0.246 |
| Rank 2 percula – Rank 3 peri | 0.52504   | 0.1333| 86.6 | 3.881 | 0.007 |
| Rank 2 percula – Rank 4 peri | 0.84013   | 0.1333| 86.6 | 6.211 | <0.0001|
| Rank 2 percula – Rank 5 peri | 1.00045   | 0.1724| 124.3| 5.802 | <0.0001|
| Rank 3 percula – Rank 4 percula | 0.31509   | 0.0901| 108 | 3.499 | 0.023 |
| Rank 3 percula – Rank 5 percula | 0.47541   | 0.1395| 118.5| 4.309 | 0.029 |
| Rank 3 percula – Rank 1 peri | 0.06702   | 0.1333| 86.6 | 0.495 | 1     |
| Rank 3 percula – Rank 2 peri | 0.00564   | 0.1333| 86.6 | 0.042 | 1     |
| Rank 3 percula – Rank 3 peri | 0.26534   | 0.1009| 31.8 | 2.629 | 0.246 |
| Rank 3 percula – Rank 4 peri | 0.58043   | 0.1333| 86.6 | 4.291 | 0.002 |
| Rank 3 percula – Rank 5 peri | 0.74075   | 0.1724| 124.3| 4.296 | 0.001 |
| Rank 4 percula – Rank 5 percula | 0.16032   | 0.1395| 118.5| 1.15  | 0.978 |
| Rank 4 percula – Rank 1 peri | −0.24808  | 0.1333| 86.6 | −1.834| 0.072 |
| Rank 4 percula – Rank 2 peri | −0.30945  | 0.1333| 86.6 | −2.288| 0.048 |
| Rank 4 percula – Rank 3 peri | −0.04975  | 0.1333| 86.6 | −0.368| 1     |
| Rank 4 percula – Rank 4 peri | 0.26534   | 0.1009| 31.8 | 2.629 | 0.246 |
| Rank 4 percula – Rank 5 peri | 0.42566   | 0.1724| 124.3| 2.469 | 0.296 |
| Rank 5 percula – Rank 1 peri | −0.04839  | 0.1719| 123.9| −2.376| 0.034 |
| Rank 5 percula – Rank 2 peri | −0.46977  | 0.1719| 123.9| 2.733 | 0.0172|
| Rank 5 percula – Rank 3 peri | −0.21007  | 0.1719| 123.9| −1.222| 0.967 |
| Rank 5 percula – Rank 4 peri | 0.10502   | 0.1719| 123.9| 0.611 | 1     |
| Rank 5 percula – Rank 5 peri | 0.26534   | 0.1009| 31.8 | 2.629 | 0.246 |
| Rank 5 peri – Rank 1 peri | −0.06137  | 0.0901| 108 | −0.682| 1     |
| Rank 5 peri – Rank 2 peri | 0.19832   | 0.0901| 108 | 2.202 | 0.462 |
| Rank 5 peri – Rank 3 peri | 0.51342   | 0.0901| 108 | 5.701 | <0.0001|
| Rank 5 peri – Rank 4 peri | 0.67373   | 0.1395| 118.5| 4.831 | 0.0002|
| Rank 5 peri – Rank 5 peri | 0.2597    | 0.0901| 108 | 2.884 | 0.123 |
| Rank 5 percula – Rank 4 peri | 0.57497   | 0.0901| 108 | 6.383 | <0.0001|
| Rank 5 percula – Rank 5 peri | 0.73511   | 0.1395| 118.5| 5.271 | <0.0001|
| Rank 5 peri – Rank 4 peri | 0.31509   | 0.0901| 108 | 3.499 | 0.023 |
| Rank 5 peri – Rank 5 peri | 0.47541   | 0.1395| 118.5| 3.409 | 0.029 |
| Rank 4 peri – Rank 5 peri | 0.16032   | 0.1395| 118.5| 1.15  | 0.978 |
Figure A1. Behavioural frequencies (behaviours/min) for the anemonefishes *A. percula* and *A. perideraion* in Kimbe Bay, Papua New Guinea. Central bar: median; boxes: lower and upper quartiles; whiskers: ±1.5 × interquartile range; dots: outliers. Observed behaviours were pooled into four categories (see Table A2).