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# The role of ecological and social constraints in social evolution of coral reef fishes

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BOSTON UNIVERSITY  
GRADUATE SCHOOL OF ARTS AND SCIENCES

Dissertation

**THE ROLE OF ECOLOGICAL AND SOCIAL CONSTRAINTS IN SOCIAL  
EVOLUTION OF CORAL REEF FISHES**

by

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**THE ROLE OF ECOLOGICAL AND SOCIAL CONSTRAINTS IN SOCIAL  
EVOLUTION OF CORAL REEF FISHES**

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**ABSTRACT**

Identifying the factors that promote social evolution, and investigating how they influence the costs and benefits of living in a group, is a major goal of evolutionary biology. My dissertation research generates a novel framework for understanding the effect of alternative options, i.e. outside options (ecological constraints) and inside options (social constraints), in social evolution using two marine systems, the clown anemonefish *Amphiprion percula* and the humbug damselfish *Dascyllus aruanus*. In the first part of my dissertation, I explored how ecological and social constraints influence the evolution of non-breeding strategies in *A. percula*. Using three manipulation experiments in the wild, I explored why clownfish non-breeders engage in peaceful cooperation, waiting to inherit breeding positions, rather than engaging in one of two alternative non-cooperative options: i) the outside option, i.e. leaving to breed elsewhere; and ii) the inside option, i.e. contesting to breed at home. I demonstrated that clownfish non-breeders will disperse, when ecological constraints (risk of mortality during dispersal) are experimentally weakened, and will contest, when social constraints (risk of eviction during contest) are experimentally relaxed. In the second part of my dissertation

I broadened our understanding of the effect of alternative options in social evolution using the humbug damselfish *D. aruanus*, a species with a more complex social system. First, I developed a new tagging technique and examined the survival time of the tags at various positions on the body of the fish. Next, I developed an essential method for data collection and behavioral observations of *D. aruanus* by i) generating an ethogram describing the behavioral repertoire of the species and ii) investigating how these fish responds to the presence of human observers and how the method of data collection may affect the quality of behavioral data. Subsequently, using a manipulation experiment in the wild, I examined if the intensity of conflict and cooperation vary with respect to the ecological and social context. I characterized habitat surrounding groups and investigated whether social context (i.e. fish size, size ratios, and group size) or ecological context (i.e. focal coral size, neighboring corals size, and number of neighboring groups) influenced the intensity of conflictive and cooperative interactions. Then, I experimentally increased individuals' outside options by adding a vacant coral and measured the intensity of conflictive and cooperative interactions in the presence and absence of the outside option. I showed that the effect of the experimental manipulation of ecological constraints on conflictive interactions in *D. aruanus* depends on the current ecological and social context surrounding groups and individuals, while the effect on cooperative interactions depends only on the ecological context. Together, the results of my dissertation research reveal how, in both *A. percula* and *D. aruanus*, ecological and social constraints act in concert influencing social dynamics and group stability, providing new insights into social evolution. Doing so highlights the importance of studying the evolution of

cooperation and non-breeding behaviors in taxonomically varied social systems, to generate a more broad and solid framework for understanding social evolution.



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## LIST OF ABBREVIATIONS

|       |   |
|-------|---|
| CA    | Central Anterior                              |
| CM    | Central Medium                                |
| CP    | Central Posterior                             |
| CI    | Confidence Interval                           |
| DA    | Dorsal Anterior                               |
| DF    | Degree of Freedom                             |
| DM    | Dorsal Medium                                 |
| DP    | Dorsal Posterior                              |
| EDA   | Exploratory Data Analysis                     |
| GLM   | Generalized Linear Model                      |
| GLMM  | Generalized Linear Mixed Model                |
| HP    | Hypothesis                                    |
| IACUC | Institutional Animal Care and Use Committee   |
| ID    | Identity                                      |
| SCUBA | Self-Contained Underwater Breathing Apparatus |
| SD    | Standard Deviation                            |
| SE    | Standard Error                                |
| SL    | Standard Length                               |
| VA    | Ventral Anterior                              |
| VM    | Ventral Medium                                |
| VP    | Ventral Posterior                             |



## CHAPTER ONE

### Introduction

Understanding the evolution of cooperation and non-breeding behaviors in animal social groups is a major goal for evolutionary biology. Cooperative breeding theory provides a rich framework to study social evolution and investigate the factors that influence the costs and benefits of remaining in a group as a subordinate non-breeder. However, in the last 60 years, most theoretical formulations of social system evolution were inspired by studies of terrestrial birds, insects and mammals (e.g., Woolfenden & Fitzpatrick, 1984; Emlen & Wrege, 1988; Keller & Reeve, 1994; Clutton-Brock, 2002; Kokko et al., 2002; Griffin & West, 2003; Shen et al., 2017). Therefore, for a long time, despite the numerous excellent studies testing key concepts of the theory, we lacked a wider taxonomic and environmental perspective for the study of social evolution, which led to the strong bias of considering high relatedness and kin selection (i.e. the presence of related and helpful subordinates) to be critical for social evolution (Bourke, 2011; Rubenstein & Abbott, 2017). However, recent consideration of other taxa, especially marine taxa, showed the occurrence of cooperation and non-breeding behaviors also in social groups composed of non-relatives, slowly revealing that cooperative breeding theory can and should encompass a continuum of taxonomically varied cooperative social systems (Sherman et al., 1995; Hing et al., 2017; Rubenstein & Abbott, 2017).

Interestingly, some marine fishes live in social groups that present a striking resemblance to the simple eusocial societies of cooperatively breeding mammals, birds,

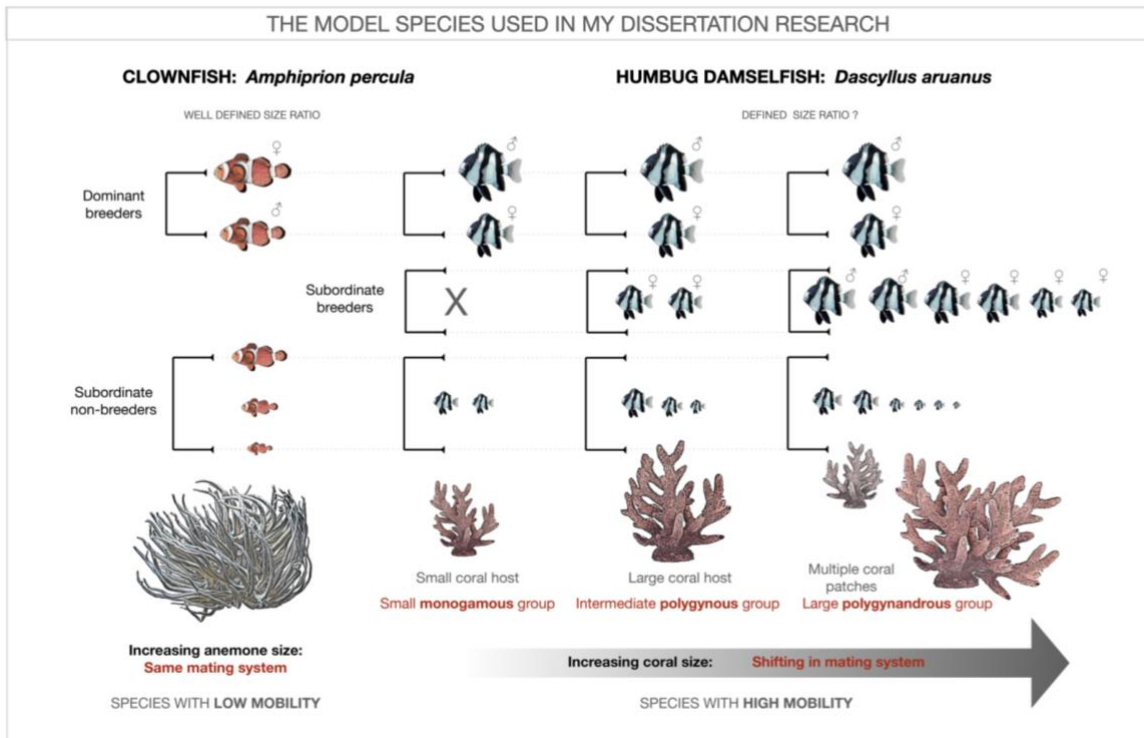
freshwater fishes and invertebrates (Taborsky & Limberger, 1981; Emlen, 1991; Sherman et al., 1995; Duffy et al., 2000; Buston, 2002; Wong, 2007; Bourke, 2011; Wong & Buston, 2013) but without the organization in family groups typical of most terrestrial cooperative breeding species (Emlen, 1995). In fact, in the vast majority of marine fishes, generally larval dispersal breaks up kin and prevents kin selection from operating (Victor, 1984; Leis, 1991; Shanks, 2009) although recent studies (D'Aloia & Neubert, 2018; D'Aloia et al., 2018; Buston et al., 2009; Rueger et al., 2020, 2021) have shown that in certain marine species with limited dispersal, interestingly, there is a possibility for weak kin selection. Additionally, in marine fish societies, cooperation can take very different forms than in most terrestrial cooperative breeding species. In fact, in contrast to terrestrial systems and freshwater fish (Riedman, 1982; Wong & Balshine, 2011), alloparental care (i.e. the act of providing care directed by group members for offspring other than their own), is rarely present in marine fishes. Instead, subordinates can cooperate by modifying their growth to remain small and reduce conflict with dominant group members (i.e. peaceful cooperation; Buston, 2003b; Wong et al., 2007; Branconi et al., 2020), or defending and maintaining the territory (Mariscal, 1966; Iwata & Manbo, 2013). Hence, marine societies offer a unique opportunity for exploring other equally plausible evolutionary forces to test the robustness of current theories and generate new insights and wider perspectives for the study of social evolution (Rueger et al., 2021).

Currently, despite the huge amount of work that has been done to identify and investigate the different factors promoting group living and cooperative actions, there is still debate regarding the relative importance of the various evolutionary forces in play

(Buston & Zink, 2009; Cant & Johnstone, 2009), suggesting that there may not be a general explanation but rather a dynamic and taxonomically varied combination of factors influencing the formation, maintenance and transformation of social groups (Hing et al., 2017). The theory of ecological constraints (Emlen; 1982) or “outside option principle” stresses that cooperative options will be favored when the habitat is saturated (i.e., there are no high-quality habitat vacancies) or it is too dangerous to move between patches of suitable habitat (i.e., there is a high risk of predation). That is, cooperative actions will be favored when ecological constraints are harsh or there are poor options outside the group (Emlen, 1982; Cant & Johnstone, 2009; Shen et al., 2017). This theory also predicts that if individuals’ outside options increase, then there is more conflict between group members and groups become less stable. While many studies have demonstrated that outside options can influence group stability (Komdeur, 1992; Wong, 2010), few have verified the effect of outside options on the intensity of conflict in terrestrial systems, and none have done so in marine systems. This is surprising given that variation in outside options could lead to variation in individual social interactions, and variation in individual social interactions could influence the structure and function of social groups, both of which could influence individual fitness (Silk et al., 2009; Oh & Badyaev, 2010). The theory of social constraints (Buston & Wong, 2014) or “inside option principle” stresses that cooperative options will be favored when there are some social constraints, such as the occurrence of size-based dominance hierarchies and a credible threat of eviction as punishment. More precisely, selection should favor dominant individuals that evict rather than tolerate subordinates who became too close in

size (otherwise the dominant would risk being overthrown); on the other hand, selection should favor subordinate individuals that i) regulate their growth and size (because of the threat of eviction) and ii) wait to inherit rather than contest for a breeding position (because of the size differences maintained within a group). That is, cooperative actions will be favored when social constraints are harsh or there are poor options inside the group (Muthoo, 2000; Buston & Zink, 2009). This theory also predicts that if individuals' inside options increase, then there is more conflict between group members and groups become less stable. However, limited work has been done to investigate how social constraints influence the evolution of social systems.

The overarching goal of my dissertation research is to investigate the effect of alternative options (i.e. ecological and social constraints) in social evolution using two marine systems: the clown anemonefish *Amphiprion percula* (a species with low mobility, small group size, and simple social system) and the humbug damselfish *Dascyllus aruanus* (a species with high mobility, large group size and complex social system). These two species were chosen because their social organization present remarkable similitudes to the simple terrestrial eusocial societies (Emlen, 1991; Sherman et al., 1995; Buston, 2002; Wong, 2007; Wong & Buston, 2013) and because they have four main characteristics that make them tractable systems for addressing the goal of my research (Figure 1.1).



**Figure 1.1** Schematic of the social organization of *Amphiprion percula* (left) and *Dascyllus Aruanus* (right). X denotes no individuals present in that rank.

First, it is possible to locate, map and follow individual fish over time: by phenotypic variation in *A. percula* (Buston, 2004a) and by tagging each individual in *D. aruanus* (Branconi et al., 2019a). Second, a single group of unrelated fish (Buston et al., 2007; Buston et al., 2009) of 2-6 individuals for *A. percula* and 2-30 individuals for *D. aruanus* is organized by size-based dominance hierarchies in which only the biggest individuals are the breeders (Buston, 2003a; Buston & Cant, 2006; Holbrook et al., 2000; Sale, 1972; Coates, 1980; Figure 1.1). The size-based dominance hierarchy likely reduces the payoff associated with contesting for breeding positions and therefore pursuing alternative actions inside the group, suggesting that inside options play a role in social

evolution in these systems. Third, each group lives in close association with a cnidarian host (a coral patch for *D. aruanus* and an anemone for *A. percula*) which provides protection from predators (Sale, 1972; Buston & Cant, 2006; Mariscal, 1970; Fautin, 1992; Forrester, 1990). The risk of moving between anemones and corals likely reduces the payoff associated with leaving the group and therefore pursuing alternative actions outside the group, suggesting that outside options play a role in social evolution of these systems. Finally, there is lots of natural variation in ecological and social variables which will enable experimental tests of the predictions regarding outside and inside options (Buston & Wong, 2014; Forrester, 1990; Wong et al., 2012).

The first part of my dissertation sets the foundations of my research project, outlining the basic understanding of the role of alternative options in the social evolution of the clown anemonefish *Amphiprion percula*, the main focus of the first two decades of social evolution research in the marine realm. More specifically, in Chapter 2 I explored why clownfish non-breeders engage in peaceful cooperation, waiting to inherit breeding positions, rather than engaging in one of two alternative non-cooperative options: i) the outside option (i.e. leaving to breed elsewhere); and ii) the inside option (i.e. contesting to breed at home). Therefore, to test the hypothesis that non-breeding individuals do not disperse to breed elsewhere because of strong ecological constraints in the form of risk of mortality during dispersal, I experimentally tested the critical prediction that non-breeding individuals will disperse when the risk of moving between anemones is reduced. Risk was manipulated by presenting alternative anemones in succession at a distance of

0.5 m and 5.0 m from focal groups. Then, to rule out alternative hypothesis (i.e. non-breeders do not leave to breed elsewhere because there are benefits in stable relations with other fish within the group and/or with the anemone; there is limited plasticity of movement in clownfish) I conducted a second experiment testing the critical prediction that non-breeders will not return to their home anemone when the risk of moving between anemones is increased. Risk was manipulated by relocating non-breeding individuals inside alternative anemones placed in succession at a distance of 0.5 m and 5.0 m from their home anemone. Finally, to test the hypothesis that non-breeding individuals do not contest for breeding positions because of strong social constraints in the form of evictions of non-cooperative individuals, I experimentally tested the critical prediction that non-breeding individuals will contest for breeding positions when the probability of winning a contest is increased. The probability of winning a contest was manipulated by the removal of rank 3 non-breeding individuals from the focal groups and the introduction of rank 3 of a different size, either a few millimeters smaller or bigger than the original rank 3. Results from Chapter 2 served as groundwork from which I extended the framework for the study of social evolution to another marine fish, *D. aruanus* and, more generally, provide insight on how ecological and social constraints combine to promote the evolution of non-breeding strategies and the formation of complex social groups.

The second part of my dissertation broadens our understanding of the effect of alternative options in social evolution using the humbug damselfish *D. aruanus*, a species with a very complex social system. In *D. aruanus*, in fact, unlike in *A. percula*, groups

have weakly defined size-based dominance hierarchies (Coates, 1980; Cole, 2002; Asoh, 2003; Wong et al., 2012) and their mating system is highly plastic, shifting from monogamy to polygyny to polygynandry as group and coral size increases (Fricke, 1980; Wong et al., 2012). In addition, sometimes groups have multiple coral hosts within their territories and fish move between them, both on their own and in groups (Mann et al., 2014). Given the high mobility of this fish, individual identification is very important for the study of social dynamics in this species; however, standard tagging methodologies (e.g., phenotypic variation, beads and PVC tags, fluorescent elastomer injections) are ineffective on this fish (Branconi et al., 2019a). Therefore, in Chapter 3 I developed a new method to temporarily tag *D. aruanus* using colored plastic films and topical surgical glue and I examined the survival time of the tags at various positions on the body of the fish. Importantly, these films are easily detectable both directly by human observers and indirectly by video/photo-cameras and could be applied successfully also to other fishes and aquatic organisms (e.g., amphibians) in both marine and freshwater ecosystems. Then, in Chapter 4 I developed an essential method for data collection and behavioral observations of *D. aruanus*. More specifically I developed an ethogram describing the behaviors of the species and investigated how *D. aruanus* responds to the presence of human observers (effect of scuba diver presence-absence) and how the method of data collection (directly by a scuba diver v. indirectly via video camera) may affect the quality of behavioral data. The results from Chapter 4 provide a foundation for future behavioral research on *D. aruanus* and other fishes where scuba divers or video cameras are the prevalent means of data collection. In Chapter 5, by means of the methods that I



developed in Chapters 3 and 4, I used the humbug damselfish *D. aruanus* to test the hypotheses that intensity of conflict and cooperation vary with respect to the ecological constraints under natural and experimental conditions. I characterized habitat surrounding groups and investigated whether ecological context (i.e. focal coral size, neighboring corals size and number of neighboring groups) influenced social behaviors (i.e. conflictive and cooperative interactions) under natural/baseline conditions. Then, I experimentally increased individuals' outside options by adding a vacant coral (i.e. the outside option) and measured social behaviors (i.e. conflictive and cooperative interactions) in the presence and absence of the outside option. Furthermore, to explore the role of social constraints in this species, I tested the hypothesis that intensity of conflict and cooperation vary with respect to social context. First, we investigated whether there is a well-defined, nonrandom, size ratio between individuals adjacent in rank, as seen in some other coral reef fishes (e.g., Buston & Cant 2006; Wong et al. 2007). Then, after characterizing social constraints as relaxed (i.e. poorly defined, random), I investigated whether social context (i.e. size ratios between individuals, fish size and group size) influenced social behaviors (i.e. conflictive and cooperative interactions) under natural and experimental conditions. Results of Chapter 5 expand our understanding of the evolution and maintenance of sociality in a species with a complex social system and test the generality of theory previously investigated in *A. percula*.

Together, results from my dissertation provide a solid framework for understanding how ecological and social constraints influence the evolution and maintenance of social groups in marine systems and, ultimately, contribute to our ability

to predict how wild individuals and populations will respond to changing ecological and social environments. More generally, my dissertation research highlights the utility of using marine species as model systems for testing the generality and advancing of our current understanding of the mechanism involved in the evolution of complex social groups.

## CHAPTER TWO

### **Ecological and social constraints promote group living in the clown anemonefish**

#### **2.1 Abstract**

Individuals that forgo their own reproduction in animal societies represent an evolutionary paradox because it is not immediately apparent how natural selection can preserve the genes that underlie non-breeding strategies. Cooperative breeding theory provides a solution to the paradox: non-breeders benefit by helping relatives and/or inheriting breeding positions; nonbreeders do not disperse to breed elsewhere because of ecological constraints. However, the question of why non-breeders do not contest to breed within their group has rarely been addressed. Here, we use a wild population of clownfish (*Amphiprion percula*), where non-breeders wait peacefully for years to inherit breeding positions, to show non-breeders will disperse when ecological constraints (risk of mortality during dispersal) are experimentally weakened. In addition, we show non-breeders will contest when social constraints (risk of eviction during contest) are experimentally relaxed. Our results show it is the combination of ecological and social constraints that promote the evolution of non-breeding strategies. The findings highlight parallels between, and potential for fruitful exchange between, cooperative breeding theory and economic bargaining theory: individuals will forgo their own reproduction and wait peacefully to inherit breeding positions (engage in cooperative options) when there are harsh ecological constraints (poor outside options) and harsh social constraints (poor inside options).

## 2.2 Introduction

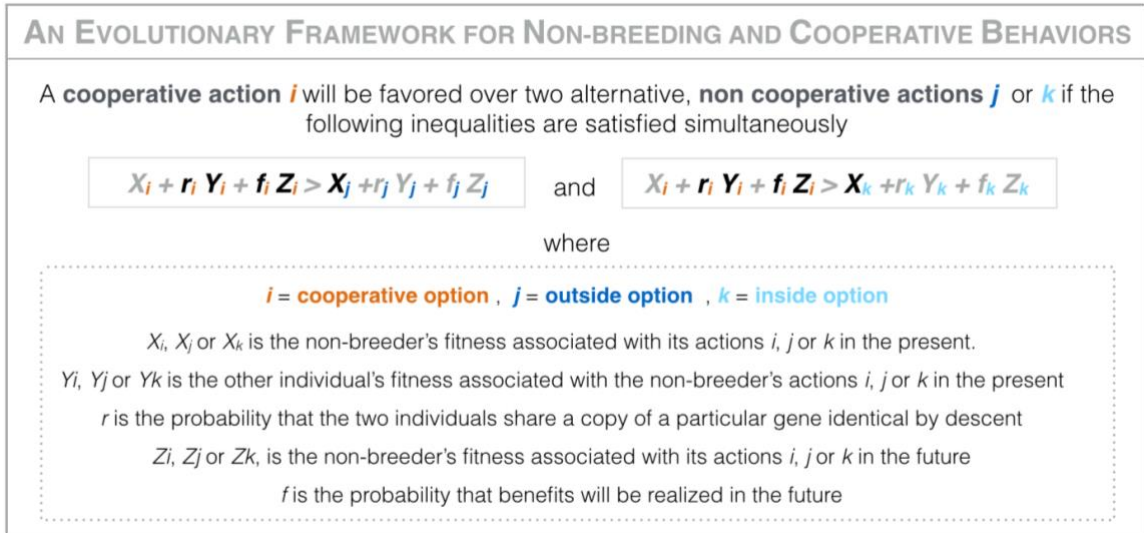
The evolution of non-breeding and cooperative behaviors, and the formation of social groups, can be readily understood using Hamilton's inequality (Emlen, 1981; Hamilton, 1964) (Fig. 2.1). Individuals will be more likely to forego their own reproduction and engage in cooperative behaviors, if there is high relatedness between group members (Hamilton, 1964; West-Eberhard, 1975) such that they can pass on their genes by helping their relatives in the present (Emlen 1989, Reeve et al., 1990; Brouwere et al., 2005) and/or if there is a high probability of inheriting a breeding position (Wiley & Rabenold, 1984; Kokko & Johnstone, 1999) such that they will pass on their genes in the future (Woolfenden & Fitzpatrick, 1978; Creel & Waser, 1994; Balshine-Earn et al., 1998) (i.e., the left hand side of the *Hamilton's inequality* is high). Also, individuals will be more likely to forego their own reproduction and engage in cooperative behaviors, if there are strong ecological constraints (Emlen, 1982; Hatvhwell & Ko,oder, 2000) such that there are no opportunities for breeding outside of the group (Komodeur, 1992; Faulkes et al. 1997; Bermüller et al., 2005) and/or if there are strong social constraints such that there are no immediate opportunities for breeding inside the group (Koenig & Pitelka, 1979; Cant et al., 2010; Dey et al., 2015) (i.e., the right hand side of the *Hamilton's inequality* is low). While there is extensive observational and experimental evidence demonstrating that high relatedness, future benefits and ecological constraints help explaining non-breeding behaviors in animal societies, relatively limited work has been done to investigate the roles of social constraints (Cant, 2011). Furthermore, there is a real need to broaden the diversity of social taxa and types of cooperative behaviors

considered, so that we may better understand the drivers of social group formation across taxa and along the continuum from simple to complex eusocial systems (Sherman et al., 1995; Hing et al., 2017).

The clown anemonefish (*Amphiprion percula*) lives in social groups composed of a breeding pair and zero to four non-breeding individuals on the coral reefs of Papua New Guinea (Fricke & Fricke, 1977). Non-breeders cooperate by remaining small and not inflicting costs on their dominants (Buston, 2003a; Buston & Balshine, 2007), but why they engage in such peaceful cooperation remains untested (Buston & Balshine, 2007; Kokko et al., 2002). Group members are not related (Buston et al., 2007) and non-breeders do not provide alloparental care (Buston, 2004b) but they do inherit the territory within which they reside following the death of the breeders (Buston, 2004a). Each group is confined to a sea anemone (*Heteractis magnifica*) that affords protection from predators (Marsical, 1970; Verwey, 1930; Fautin, 1991; Elliott et al., 1995). However, every anemone of the reef is occupied (Fautin, 1992; Elliott et al., 2001; Buston, 2003a), because there is high recruitment rate (due to a constant rain of larval settlers that disperse from their natal anemones from distances up to 120 km; Buston, 2003a; Alamany, 2017), and low mortality rate (Buston, 2003a; Buston & Garcia, 2007; Buston, 2003c). In addition, it is risky to move between anemones, because clownfish are poor swimmers and can be preyed upon (Marsical, 1970; Verwey, 1930; Elliott et al., 1995; Moyer & Nakazono, 1978). Taken together, habitat saturation and risks of movement likely reduce the payoff associated with leaving to breed elsewhere, suggesting that ecological constraints play a role in social group formation. Within each group there is a

size-based dominance hierarchy (Buston, 2003a) where the female is the largest (rank 1), the male is second largest (rank 2) and the non-breeders get progressively smaller (ranks 3–6); if the female of the group dies, then the male changes sex and becomes the new female (clownfish are protandrous hermaphrodites; Fricke & Fricke, 1977; Moyer & Nakazono, 1978), and the largest non-breeder becomes the new male (Buston, 2004b). Within the size hierarchy subordinates tend to be 80% of the size of their immediate dominants (Buston, 2006). This factor likely reduces the payoff associated with contesting for breeding positions, suggesting that social constraints also play a key role in social group formation.

The aim of this study is to investigate why clownfish non- breeders engage in the cooperative option, waiting peacefully in social groups to inherit breeding positions, rather than engaging in one of two, alternative, non-cooperative options: (i) the outside option—leaving to breed elsewhere; and ii) the inside option— contesting to breed at home (Fig. 2.1).



**Figure 2.1** An evolutionary framework for non-breeding and cooperative behaviors based on Hamilton's inequality. The evolution of non-breeding and cooperative behaviors depends on the expected fitness associated with engaging in non-breeding and cooperative actions (the cooperative option) relative to alternative non-cooperative actions outside the group (the outside option) or inside the group (the inside option). The cooperative option can be favored by selection because of its beneficial effects on kin (e.g., via helping relatives) and in the future (e.g., via territory inheritance) and because of the low expected fitness associated with the outside option (e.g. due to ecological constraints) and the inside option (e.g., due to social constraints). In general, when we are trying to explain the evolution of non-breeding strategies,  $X_i$  on the left hand side and  $r_j Y_j$ ,  $f_j Z_j$ ,  $r_k Y_k$ ,  $f_k Z_k$  on the right hand side (terms in light gray) are considered to be zero or trivially small compared to other terms (terms in black).

## 2.3 Methods

### 2.3.1 Study population

We studied the clown anemonefish *Amphiprion percula* in Kimbe Bay, Papua New Guinea, from June to September 2018. All work was conducted using SCUBA at depths up to 20 meters. We located 186 magnificent sea anemones (*Heteractis magnifica*) on 12 inshore reefs near Mahonia Na Dari Research and Conservation Centre. Each anemone was occupied by a single group of *A. percula*. Groups consisted of a breeding

pair and zero to three non-breeders. Individuals were identified based on natural variation in their color markings.

### *2.3.2 Anemone acquisition*

We surveyed the anemone population and identified a small number of anemones that were movable e.g., attached to small rocks or only loosely attached to the hard substrate. These anemones were collected and used as alternative anemones for the ecological constraints experiments. For these experiments, alternative anemones were placed at a distance of 0.5 m or 5.0 m from the focal anemones with a clear line of sight between the two. Fish have been shown to locate anemones using chemical and visual cues at these distances (Fautin, 1991; Elliott et al., 1995; Dixon et. al., 2008; Dixon et al., 2014). During the experiment, unused fish from the alternative anemones were kept in the laboratory at Mahonia Na Dari Research and Conservation Center; at the end of the experiment, all fish and anemones were returned to their original location].

### *2.3.3 Fish measurement*

In June, we captured all fish using hand nets, placed them inside clear plastic bags, laid them against a slate, and measured their standard length to the nearest 0.1 mm using calipers. This entire procedure was conducted underwater and all individuals were returned to their anemone within a few minutes. Individuals were ranked (1–5) based on their size relative to other individuals within the anemone, with the largest being ranked 1. Rank 1 was designated as the female, rank 2 the male, and ranks 3–5 as non-breeders.



We monitored the fish population to determine which groups were breeding. Breeding was readily detectable because the male spends much of his time caring for the eggs.

#### *2.3.4 Statistical analysis*

For the first ecological constraints experiment, to test the hypothesis that the likelihood of rank 3 non-breeders dispersing will depend on the classes of alternative anemones (empty anemones or anemones with a breeding male), we used one Fisher's exact test for contingency tables. Specifically, at a distance of 0.5 m, we tested whether the number of rank 3 non-breeders that dispersed from their focal anemone to empty anemones ( $n = 16$ ) differed from the number of rank 3 non-breeders that dispersed from their focal anemone to anemones with a breeding male ( $n = 16$ ).

For the second ecological constraints experiment, to test the hypothesis that likelihood of rank 3 non-breeders returning home will depend on the classes of alternative anemones and their distances from the focal anemones, we used four Fisher's exact tests for contingency tables. First, at a distance of 0.5 m, we tested whether the number of rank 3 non-breeders that returned home from empty anemones ( $n = 16$ ) differed from the number that returned home from anemones with a breeding male ( $n = 16$ ). Second, we conducted an equivalent test at 5.0 m ( $n = 16$  for each treatment). Third, for alternative anemones that were empty, we tested whether the number of rank 3 non-breeders that returned home from 0.5 m ( $n = 16$ ) differed from the number that returned home from 5.0 m ( $n = 16$ ). Fourth, we conducted an equivalent test for alternative anemones with a breeding male ( $n = 16$  for each treatment).

For the social constraints experiment, to test the hypothesis that bigger introducees (rank 3", weaker social constraints), but not smaller introducees (rank 3', stronger social constraints), will contest for breeding positions and will be evicted by the breeding pair, we used one Fisher's exact test for contingency tables. Specifically, we tested whether the number of rank 3" that were evicted ( $n = 16$ ) differed from the number of rank 3' that were evicted ( $n = 16$ ).

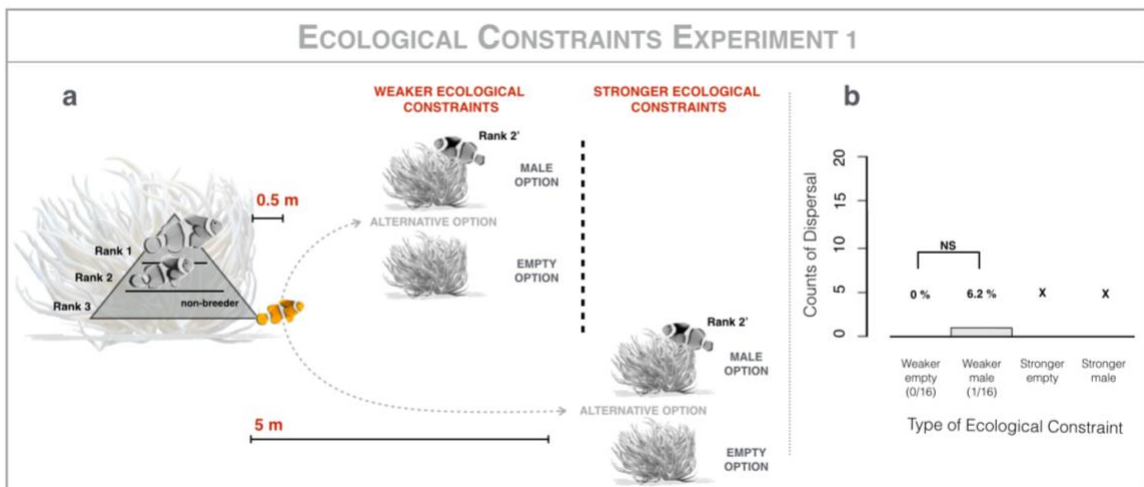
All analyses were done in R v. 3.4.2 'Short Summer'.

## **2.4 Results**

### *2.4.1 Ecological constraints experiment #1*

To test the hypothesis that non-breeding individuals do not disperse to breed elsewhere because of strong ecological constraints in the form of risk of mortality during dispersal, we experimentally tested the critical prediction that non-breeding individuals will disperse when the risk of moving between anemones is reduced. Risk was to be manipulated by presenting alternative anemones in succession at a distance of 0.5 m and 5.0 m from 32 focal groups (Fig. 2.2a). To explore the effect of variation in the alternative option, two classes of anemones were used: empty anemones ( $n = 16$ ) or anemones with a breeding male significantly larger than the focal non-breeder ( $n = 16$ ). Focal anemones were assigned to one of the two options at random. The two classes of anemone represent different potential outcomes for the focal non-breeder: if it were to disperse to the empty anemone, it would become the breeding female, and would have to

wait for a new recruit to breed; if it were to disperse to the anemone with a breeding male, it would become the breeding male, and would have to wait for the resident breeding male to change sex to breed. Focal groups all had at least one non-breeder. We left alternative anemones alongside the home anemones for 2 days, to allow the focal non-breeder sufficient time to make a choice. The morning of the third day, we recorded whether focal non-breeders (rank 3) had moved to the alternative anemone. Focal non-breeders dispersed to the alternative option placed at 0.5 m in only one out of 32 cases (Fig. 2.2b). Because so few non-breeders moved even 0.5 m and because (i) studies on fish with similar social systems indicated that likelihood of movement declined rapidly as a function of distance (Wong, 2010) and (ii) previous studies on clownfish have demonstrated that they don't move greater distances in response to naturally occurring vacancies (Buston, 2004b; Buston, 2003a), we did not present the 5.0 m option. Our result supports the hypothesis that non-breeders do not disperse to breed elsewhere because of the risks associated with moving even short distances. However, this result could also provide support for two alternative hypotheses: non-breeders do not disperse because their home anemone confers higher expected reproductive success than the presented alternative, possibly because there are some benefits in stable cooperative relations with other fish within the group or stable mutualistic relationship with the anemone (Kokko et al., 2001); non-breeders do not disperse because there is limited plasticity of movement in clownfish (i.e., moving from their home anemone is not in their behavioral repertoire), just as in many (Bourke, 2011), but not all (Nonacs, 2017; Grinsted & Field, 2017), social insects.

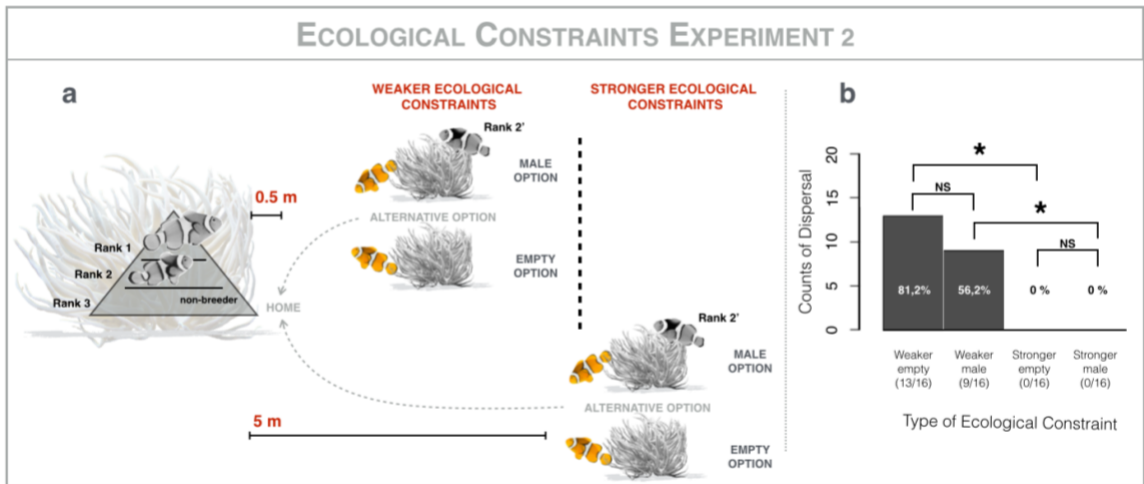


**Figure 2.2** Ecological constraints experiment #1. **a.** Methods: presentation of two classes of alternative anemones (empty anemones,  $n = 16$ ; anemones with a breeding male,  $n = 16$ ) at a distance of 0.5 m (weaker ecological constraints) and 5 m (stronger ecological constraints) from 32 focal groups. **b.** Results: comparison of the counts of dispersal of rank 3 non-breeding individuals by type of ecological constraint: weaker-empty; weaker-male; stronger-empty; stronger-male; because so few non-breeders moved to the alternative anemone at 0.5 m, we did not present the alternative anemones at 5 m, and this is denoted by “X”; Fisher’s Exact Tests: “NS” non-significant ( $p > 0.05$ ); “\*” significant ( $p < 0.05$ ); horizontal brackets indicate pairwise comparisons. All work was conducted using *A. percula* groups inhabiting *H. magnifica* anemones, on inshore reefs near Mahonia Na Dari Research and Conservation Centre, in Kimbe Bay, Papua New Guinea, from June to September 2018, using SCUBA at depths of up to 20 meters. At the end of the experiment, all individuals were returned to their home anemones.

#### 2.4.2 Ecological constraints experiment #2

To discriminate among these three alternative hypotheses, we adjusted the experimental design, and experimentally tested the critical prediction that non-breeders will not return to their home anemone when the risk of moving between anemones is increased. We presented alternative anemones at a distance of 0.5 m and 5.0 m from 32 focal groups. Each focal group was tested for both distances, in series. As above, we started with the 0.5 m experiment because if there were no movement there, then we would not predict any movement to 5.0 m (Buston, 2004b; Buston, 2003a; Wong, 2010).

Once more, two classes of anemones were used at each distance: empty anemones ( $n = 16$ ) or anemones with a breeding male ( $n = 16$ ). In this case, however, we relocated the focal non-breeder from the home anemone to the alternative (Fig. 2.3a). We left this set-up for 2 days, to allow the focal non-breeder sufficient time to make a choice. The morning of the third day, we recorded whether the focal non-breeder had returned to its home anemone. When the alternative option was placed at 0.5 m, the focal non-breeder returned to its home anemone in 22 out of 32 cases (13/16 from the empty anemone; 9/16 from the anemone with a breeding male; Fig. 2.3b). This result rejects the hypothesis that there is limited plasticity of movement in clown-fish (i.e., movement between anemones is in their behavioral repertoire even though its rarely seen under natural conditions). Notably, there were significantly more movements in this experiment than in the first experiment (Fisher's exact test,  $p < 0.001$ ). This result supports the hypothesis that non-breeders did not disperse in the first experiment because their home anemone confers higher expected reproductive success than the alternative, though it also suggests that there may be some risk to movement even in the 0.5 m treatment because not all focal non-breeders returned home in the second experiment. Finally, when the alternative anemone was presented at 5.0m, non-breeders returned to their home anemone in zero out of 32 cases (Fig. 2.3b)—in all cases, fish remained inside the alternative anemones. This is significantly less than in the 0.5 m case (Fisher's exact test,  $p < 0.001$ ). Given that anemones tend to be tens of meters apart under natural conditions, this result supports the hypothesis that non-breeders do not disperse to breed elsewhere because of harsh ecological constraints in the form of risks of movement.

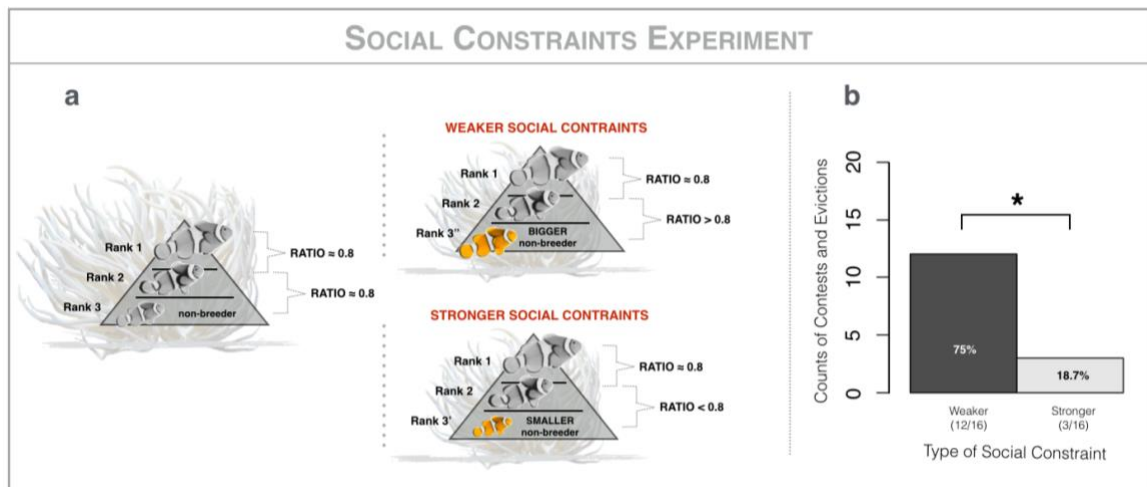


**Figure 2.3** Ecological constraints experiment #2. **a.** Methods: relocation of rank 3 non-breeding individuals inside one of the two alternative classes of anemones (empty anemones,  $n = 16$ ; anemones with a breeding male,  $n = 16$ ) at a distance of 0.5 m (weaker ecological constraints) and 5 m (stronger ecological constraints) from 32 focal groups. **b.** Results: comparison of the counts of dispersal of rank 3 non-breeding individuals by type and strength of ecological constraint: weaker-empty; weaker-male; stronger-empty; stronger-male; Fisher’s Exact Tests: “NS” non-significant ( $p > 0.05$ ); “\*” significant ( $p < 0.05$ ); horizontal brackets indicate pairwise comparisons. All work was conducted using *A. percula* groups inhabiting *H. magnifica* anemones, on inshore reefs near Mahonia Na Dari Research and Conservation Centre, in Kimbe Bay, Papua New Guinea, from June to September 2018, using SCUBA at depths of up to 20 meters. At the end of the experiment, all individuals were returned to their home anemones

### 2.4.3 Social constraints experiment

To test the hypothesis that non-breeding individuals do not contest for breeding positions because of strong social constraints in the form of evictions of non-cooperative individuals, we experimentally tested the critical prediction that non-breeding individuals will contest for breeding positions when the probability of winning a contest is increased. To test this prediction, we used 16 focal groups, all of which consisted of at least three individuals and had bred at least once in the preceding two months (Fig. 2.4a). All individuals in each focal group were caught and measured to the nearest 0.1 mm using

calipers, and the largest non-breeder (rank 3) was removed. Then, we introduced two types of rank 3 individuals to the focal group: a non-breeder less than 80% of the size of the breeding male (rank 3') or a non-breeder more than 80% of the size of their immediate dominant under natural conditions (Buston, 2006) this result supports the hypothesis that non-breeders do not contest for breeding positions because of harsh social constraints.



**Figure 2.4** Social constraints experiment. **a.** Methods: removal of rank 3 non-breeding individuals from 16 focal group and introduction of rank 3 of a different size, either a few millimeters smaller (R3'; stronger social constraints;  $n = 16$ ) or a few millimeters bigger (R3"; weaker social constraints;  $n = 16$ ) than the original rank 3. **b.** Results: comparison of the counts of contests by strength of social constraints; Fisher's Exact Tests: NS" non-significant ( $p > 0.05$ ); "\*" significant ( $p < 0.05$ ); horizontal brackets indicate pairwise comparisons. All work was conducted using *A. percula* groups inhabiting *H. magnifica* anemones, on inshore reefs near Mahonia Na Dari Research and Conservation Centre, in Kimbe Bay, Papua New Guinea, from June to September 2018, using SCUBA at depths of up to 20 meters. At the end of the experiment, all individuals were returned to their home anemones.

## 2.5 Discussion

Our findings explain why clownfish non-breeders forgo their own reproduction, resolving this evolutionary paradox. While they do not gain indirect genetic benefits from helping kin (Buston, 2004a), they do stand to gain future direct benefits by inheriting a breeding position on the death of a breeder (Buston, 2004b). Here, we show that they tolerate their non-breeding situation because harsh ecological constraints, in the form of habitat saturation and risks of movement, prevent them from successfully dispersing to breed elsewhere. Further, we show that they tolerate their non-breeding situation because harsh social constraints, in the form of well-defined size differences between individuals adjacent in rank, prevent them from successfully contesting to breed at home. Compellingly, we show that individuals will disperse and contest to better their current situation when ecological and social constraints are experimentally relaxed. In clownfish, ecological and social constraints combine to promote the evolution of non-breeding strategies, and it is necessary to understand both types of constraint to fully understand their societies.

A striking result of our first and second ecological constraints experiments is that non-breeders did not leave home when presented with an alternative anemone at 0.5 m and two-thirds of them returned home when relocated to an alternative anemone at 0.5 m. This strongly suggests some benefit of stable associations with known anemones of particular qualities, with familiar fish with whom conflicts have been resolved, and/or with a larger group of fish. Anemones vary in size and expansion behavior, which



impacts the safe foraging area available to the fish, which in turn influences the size of the fish, their investment in egg laying and parental care, and their reproductive success (Barbasch et al., 2020; Salles et al., 2020). A larger group of fish may enhance the size of their anemone, either by providing more nutrients enabling the anemone to grow more (Holdbrook & Schmitt, 2005; Cleveland et al., 2011; Porat & Chadwick-Furman, 2004), or by defending against anemone predators allowing anemones to expand their tentacles and feed more (Porat & Chadwick-Furman, 2004). Taken together, these factors could explain why non-breeders have such a strong affinity for their home anemones and the associated fish, and future studies will test these alternative hypotheses.

Another striking result of the second ecological constraints experiment and our social constraints experiment is that not all individuals responded in the same way to the manipulations: some individuals returned home while others did not; some individuals contested while others did not. One interesting hypothesis is that variation in response could be due to variation in personality traits (defined as inter-individual differences in behavior that are consistent over time and across contexts; Réale et al., 2007) of the individuals being tested. Previous studies have demonstrated the existence of personality traits (e.g., boldness, aggressiveness, shyness, sociability, and parenting behaviors) in *A. percula* (Schmiege et al., 2017; Barbasch & Buston, 2018) and its sister species *A. ocellaris* (Wong et al., 2013). It was not possible for us to test this idea, or alternative explanations for the variation in response, with our data. Future studies will investigate how individual personality traits influence individuals' decisions in clownfish societies,

and how different combinations of individuals' personality traits influence the form and function of clownfish societies.

In sum, here, we have shown that ecological and social constraints combine to promote the evolution of non-breeding strategies and the formation of complex social groups. Interestingly, this explanation for social group formation can be framed in the language of economic bargaining theory (Muthoo, 2000; Cant & Johnstone, 2009; Buston & Zink, 2009). Economic bargaining theory emphasizes that there are three options available to individuals: the cooperative option (the payoff from pursuing cooperative actions inside the group), the outside option (the payoff from pursuing non-cooperative actions outside of the group; Muthoo, 2000; Cant & Johnstone, 2009; Buston & Zink, 2009) and the inside option (the payoff from pursuing non-cooperative actions inside the group; Muthoo, 2000; Cant & Johnstone, 2009; Buston & Zink, 2009). Individuals will engage in cooperative actions when both the outside option and the inside option are poor relative to the cooperative option. Therefore, synthesizing the language of economic bargaining theory and cooperative breeding theory, reveals that individuals will forgo their own reproduction, engaging in cooperative actions such as remaining small and waiting peacefully to inherit territories, when there are poor options outside the group (strong ecological constraints) and poor options inside the group (strong social constraints). The synthesis of these two theories may lead to a fruitful exchange of ideas between fields and advances in our understanding of complex societies.

## 2.6 Acknowledgements

We are particularly grateful to James Traniello, Stephen Emlen, and Peter Nonacs for constructive feedback on earlier versions of the manuscript. We would also like to thank M. Schniedewind for assistance in the field, the staff of Mahonia Na Dari Research and Conservation Centre and Walindi Plantation Resort for logistical support in the field, and the communities of Tamare and Kilu, the traditional owners of the reefs. All work was performed with the approval of the Institutional Animal Care and Use Committee, Boston University (Protocol number: 17/001) and the Government of Papua New Guinea. The research was supported by one Sigma XI Grant in Aid of Research and one Kunz award awarded by Boston University to R. Branconi and by one NSF Doctoral Dissertation Improvement grant (grant number: IOS- 1701657), one Warren McLeod fellowship and one BU Women's Guild award awarded by Boston University to T. Barbasch.

## CHAPTER THREE

### A New Non-Invasive Technique for Temporarily Tagging Coral Reef Fishes

#### 3.1 Abstract

The ability to identify individuals is important for the success of many behavioral and ecological studies. In fishes, there is a lot of variation in body size, shape, skin thickness, behavior, and ecology, which means that any given marking/ tagging method may not work well for all species. For the Humbug Damselfish *Dascyllus aruanus*, a widely used model species of coral-reef fish, we found that standard tagging methodologies (e.g., phenotypic variation, beads and PVC tags, fluorescent elastomer injections) were ineffective as they could not be applied to the fish or easily detected by observers or from videos. In response, we developed a new method to temporarily tag *D. aruanus* using colored plastic films and topical surgical glue. These films were easily detectable both directly by human observers and indirectly by video/photo-cameras. We tested the efficacy of this new method by examining the survival time of the tags at various positions on the body. Our results showed that the optimal tag locations were dorsal anterior (with a median attachment time of 53 hours) and dorsal middle (with a median attachment time of 49 hours). Total length of fish was not a significant predictor of tag life. In sum, we demonstrate an effective new method for temporarily tagging a widely studied coral reef fish. This method could be applied to other fishes and aquatic organisms (e.g., amphibians) in both marine and freshwater ecosystems.

### 3.2 Introduction

Many studies in population ecology and behavioral ecology require individuals to be identifiable (Krebs & Davies, 1997). Generally, animal identification occurs using a means of marking or tagging (Gavin & Haas, 1989). By developing and using multiple tagging methods, scientists have been able to track individuals through time, generate estimates of mortality, and demonstrate variation in behavior amongst individuals within a population (Randall, 1962; Frusher & Hoenig, 2001; Coker et al., 2012). However, the practical difficulties of working with some taxa, notably aquatic organisms, have made acquisition of data on their biology and behavior through individual identification more challenging.

For fishes, external and internal marks and tags have been used for centuries (Rounsefell & Kask, 1945). Nevertheless, the great variation in body size, shape, skin thickness, behavior, and ecology means that the same tagging methods cannot be used for all species. Additionally, some marking/ tagging systems that are easily visible to the human eye in one context may not be as readily observable in different contexts (e.g., different light conditions and depths), and they may not work as well once photographed or filmed. Therefore, to optimize studies involving fish identification, it is essential to select the tagging system that works for the species and context under investigation.

In coral reef fishes, there are many different techniques for individual identification: phenotypic variation (Nelson et al., 1994; Booth, 1995; Buston, 2003c), genotypic variation (Puebla et al., 2007; Salles et al., 2016), anchor tags such as dart, T-

bar and spaghetti (Randall, 1962; Parker, 1990), fluorescent elastomer injection (Beukers et al., 1995; Frederick, 1997; Malone et al., 1999; Hoey & McCormick, 2006), passive integrated transponder (PIT; Holm et al., 2007; Topping & Szedlmayer, 2011), coded wire tags (Beukers et al., 1995; Verweij & Nagelkerken, 2007), bead tags (Verweij & Nagelkerken, 2007), and parasites (Grutter & Poulin, 1998; Cribb et al., 2000). These techniques vary in terms of invasiveness of procedures, duration of effectiveness, visibility, and practical application. However, for some species these methods are not appropriate.

For the widely studied coral-dwelling damselfish, *Dascyllus aruanus*, for example, there is not sufficient phenotypic variation between individuals (besides relative body size, occasional variances in lip coloration and dorsal fin pattern) and hence tagging is required (Branconi, pers. obs.). However, we found that standard methods of tagging were unsuitable for *Dascyllus aruanus* due to their specific ecology and morphology: beads and PVC tags get caught when they swim through the narrow inter-branch spaces in their host corals; fluorescent elastomer injections/tags placed in areas other than the caudal peduncle proved difficult to apply due to their thick scales and ease of bruising (Branconi, pers. obs.). Further, other attempts to tag *D. aruanus* (fluorescent elastomer injections: Booth, 2004; Coker et al., 2012; Kuwamura et al., 2016; mutilation: Sale, 1971; alcian blue dye: Mizushima et al., 2000; tetracycline staining: Forrester, 1990; liquid latex: Forrester, 1991) do not result in marks that are visible from a distance or via video recordings.

Therefore, our goal was to develop a new method for temporarily tagging this species that is easily detectable by both human observers and indirectly by video/photo-cameras. Specifically, we addressed the following three questions: i) What is the attachment time of the tags? ii) How does fish body size influence the attachment time of the tags? and iii) How does position on the body influence attachment time of the tags? In sum, we developed a new non-invasive technique for temporarily tagging coral reef fishes that is potentially broadly applicable to other aquatic species.

### **3.3 Methods**

#### *3.3.1 Natural history*

*Dascyllus aruanus* is a tropical coral-reef fish that is widespread throughout the Indo-Pacific region and lives in social groups in close association with coral colonies of certain branching corals (Sale, 1971; Holbrook et al., 2000). Within each discrete coral patch there is a single group of mostly non-relatives (Sale, 1971; Fricke, 1980; Buston et al., 2009) with average group size of eight individuals (Sale, 1972; Holbrook et al., 2000) organized into size-based dominance hierarchies (Coates, 1980; Wong et al., 2012).

#### *3.3.2 Collection and housing*

For this study, we used a population of *D. aruanus* at Lizard Island Lagoon on the northern Great Barrier Reef, Australia (14840<sup>0</sup>S, 145827<sup>0</sup>E). The study was conducted during January 2016. Two collection sites were selected: Palfrey Island (14841.764<sup>0</sup>S, 145826.890<sup>0</sup>E) and Trawler Beach (14841.060<sup>0</sup>S, 145827.662<sup>0</sup>E). At

each of these sites, we located and mapped 11 corals occupied by a single group of *D. aruanus*. Group sizes ranged from three to four fish and were determined by counting the number of resident fish on the focal host coral. Fish from each group were anesthetized using a clove oil solution (Munday & Wilson, 1997), captured with a slurp gun or hand net, and placed into zip-lock plastic bags to be transferred to the boat. Once onboard, air was put in the bags, the bags were put in a cooler, and the fish were transported to laboratory facilities at Lizard Island Research Station (the time from catching to release the fish into the aquaria was less than one hour).

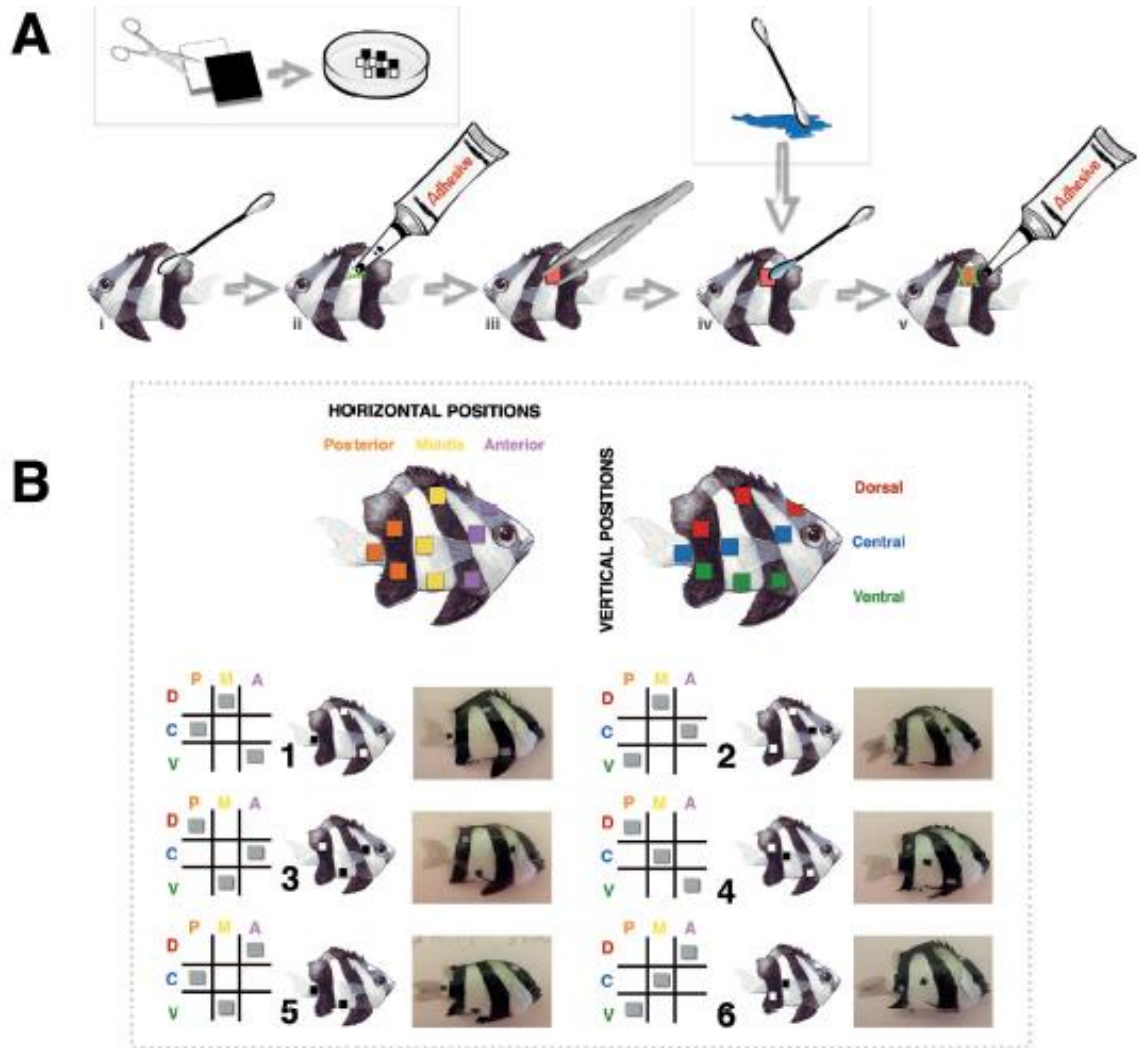
Each group of fish was placed into a rectangular plastic aquarium (68 liters; external 645x413x397 mm, internal 597x362x381 mm; Nally IH078) containing dead coral heads (coral heads of multiple species with the same morphology as the host corals commonly used by *D. aruanus*) positioned under direct natural sunlight conditions shaded by a plastic screen (11 groups of fish; 36 individuals). A flow-through system provided a continuous supply of fresh seawater (directly pumped from the reef in front of the Research Station) guaranteeing reasonable water quality, temperature, and some natural food within the aquaria. Additionally, fish were fed twice a day with dry fish pellet and living brine shrimp larvae, *Artemia salina*. All fish were held for ten days for the duration of the procedures (see below) before being returned to their original collection site.



### *3.3.3 Tagging procedure*

The plastic tags consisted of white or black plastic films (60/80 micron continuous polymeric material) cut into approximately 3x3 mm squares (Fig. 3.1A). The color of the tags (black and white) was chosen to be consistent with the natural coloration of the fish, so as not to increase the visibility of marked individuals to predators but making marked individuals easily observable under different light conditions to the human eye or to video/photo- cameras.

To tag the fish, each individual was removed from the aquaria and placed on its side on a plastic slate positioned on a wet bench with low water flow so that a thin and constant layer of water was in contact with the fish. Fish were not re- anesthetized prior to tagging. Using a cotton tip, a few scales of the fish were dried at the appropriate tag positions (Fig. 3.1A). Subsequently, a small drop of the topical tissue adhesive “GLUture” (an Octyl/Butyl cyanoacrylate blend that offers a flexible seal; Abbott Laboratories, Chicago, IL) was placed onto the dried scales and a plastic tag placed on the drop of adhesive using tweezers (Fig. 3.1A).



**Figure 3.1** (A) Graphic scheme of the tagging procedure: i) Dry the scales of the fish, scraping gently with a clean cotton tip (moving the top of the cotton tip in the same direction as the fish scales); ii) Apply on the dried area a very small drop of topical tissue adhesive; iii) Place the tag on the spot of adhesive using tweezers; iv) Using a wet cotton tip, press on the tag for few seconds (ensuring that there are no bubbles of air between the tag and the fish scales); v) Finally, apply a small amount of adhesive on the right and left extremities of the tag. This procedure requires less than a minute for each tag. (B) Graphic scheme and photo examples of the six possible combinations of the tags. Positions of the tags along the fish body (horizontal—posterior in orange, middle in yellow, and anterior in purple; and vertical—dorsal in red, central in blue, and ventral in green).

To ensure that the entire surface of the tag was touching the fish's scale (i.e., there were no air bubbles between the tag and fish scales), the tag was pressed onto the scale for few seconds using a wet cotton tip (Fig. 3.1A). A small amount of topical tissue adhesive was then placed on top of the right and left extremities of the tag (Fig. 3.1A). Six tags were placed at various defined positions on the body, three on each side (Fig. 3.1B). The placement of each tag required approximately 60 seconds. Fish were returned to the aquarium between the placement of each tag. Finally, fish total length was measured to the nearest 0.1 mm using calipers.

#### *3.3.4 Experimental design*

Each individual within each group of fish ( $n = 36$  individuals;  $n = 11$  groups) was randomly assigned a different combination of tags, of which there were six possible combinations (Fig. 3.1B). Each combination was defined by the position of three tags on both the left and right side of the fish along i) horizontal positions—subdivided into posterior, middle, and anterior and ii) vertical positions—subdivided into dorsal, central, and ventral (Fig. 3.1B). Following standard operating procedures approved by the animal ethics committee of the University of Wollongong (UOW), after application of tags, a qualitative assessment of fish behavior and health was conducted checking for any sign of stress (e.g., abnormal breathing rate, irregular movements and buoyancy, or anomalous feeding activity).

Subsequently, the presence–absence of the tags was monitored every two hours for the first three days and then every four hours for the following four days (total of

seven days). After tag loss, fish were observed for three days to assess scale replacement. To ensure that tags were not missed, each check was performed by an observer (RB) watching the fish in their aquaria, and by analyzing five minutes of video recordings taken by HERO3+ GoPro™ cameras (GoPro Inc., San Mateo, CA) fixed on tripods placed inside the aquaria.

### *3.3.5 Statistical analysis*

To generate a preliminary visual understanding of whether attachment time of tags differed based on tag position, an Exploratory Data Analysis (EDA) was performed by producing and examining three figures: i) box plots of attachment time of tag by tag position for both fish sides; ii) color gradient plot of mean attachment time for each tag location; and iii) box plots of attachment time of tag by fish size ordered according to fish size.

Subsequently, we used the observations from the EDA to inform more rigorous statistical testing. More specifically, we fitted a linear mixed-effects model to the data using the R package lme4 (R Core Team, 2014; Bates et al., 2015) to assess whether tag attachment time was influenced by position and/or fish total length. To normalize the data (based on visual inspection of QQ plots), tag attachment time was log transformed. A linear mixed-effects model was fitted with tag position and fish total length as predictors and log tag attachment time as the response. Fish ID was included as a random effect to account for lack of independence among multiple tags on the same fish. P-values were calculated using the Kenward Rogers approximation for the degrees of freedom.

### 3.4 Results

In this study, we used a total of 36 individuals (average total length = 45.2 mm, ranging from 72.6 mm to 20.2 mm) and attached a total of 216 tags. Following UOW Standard Operating Procedures, no signs of stress were visible in the fish. Within five minutes of the tagging procedure, the behavior of the marked individuals appeared normal (i.e., regular breathing rate, movements, and buoyancy). By the end of our experiment, all fish appeared healthy (i.e., normal activity and regular feeding), and the majority of fish possessed new scales as replacements for scales lost with the tags. In sum, the tagging procedure caused no apparent harm to the fish within the timeframe of the experiment.

Initial EDAs revealed that median attachment time of tags ranged widely with tag position, from a minimum of 15 hours for ventral anterior (VA) positions to a maximum of 145 hours for central middle (CM) positions (see Table 3.1 for the values of median, minimum, and maximum tag attachment time for each position). More specifically, the box plots displaying tag attachment time by tag position (Fig. 3.2A) and the color gradient plot displaying the mean attachment time of each tag location (Fig. 3.2B) suggested that optimal tag locations are dorsal anterior (DA), with a median attachment time of 53 hours (mean attachment time = 62 hours), and dorsal middle (DM), with a median attachment time of 49 hours (mean attachment time = 50 hours). On the other hand, central anterior (CA) locations and ventral posterior (VP) locations seemed to be the worst tag locations, presenting a median attachment time of 22 hours (mean

attachment time = 39 hours) and 25 hours, respectively (mean attachment time = 40 hours; Fig. 3.2A, B). The box plots also indicated that the pattern of tag life was similar on the right and left sides of the fish (Fig. 3.2A), suggesting that fish side does not influence tag attachment time. The box plots displaying tag attachment time by individual ordered by size (Fig. 3.3) were not suggestive of any effect of body size on tag attachment time.

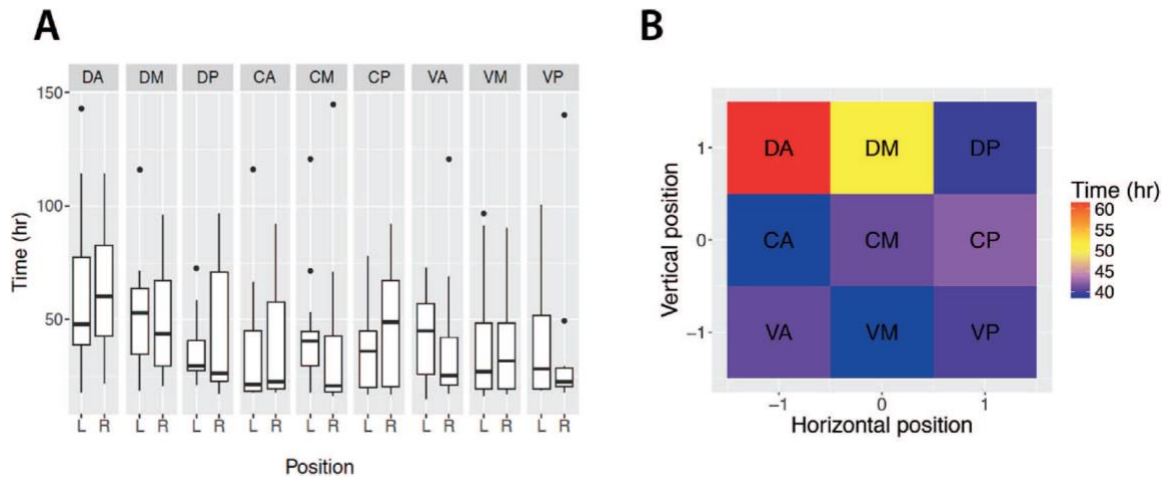
The linear mixed-effects model revealed that the dorsal anterior (DA) and dorsal middle (DM) positions had significant and large positive coefficients (0.504 and 0.284, respectively), indicating that these tag positions resulted in the longest tag life, confirming the results of our initial EDA (see Table 3.2 for the values of the model coefficients and relative estimate, standard error, 95% confidence intervals, and t- and P-values of all positions). Finally, the linear mixed-effects model revealed that fish total length was not a significant predictor of tag attachment time (Table 3.2), which is also consistent with the results of our initial EDA.

**Table 3.1** Median, minimum, and maximum tag attachment time (hr) for each position: dorsal anterior, middle, and posterior (DA, DM, DP); central anterior, middle, and posterior (CA, CM, CP); ventral anterior, middle, and posterior (VA, VM, VP).

| Tag attachment time (hr) |       |        |        |
|--------------------------|-------|--------|--------|
| Position                 | Min   | Median | Max    |
| DA                       | 18.25 | 53.00  | 143.00 |
| DM                       | 19.00 | 49.25  | 116.25 |
| DP                       | 17.50 | 29.00  | 96.75  |
| CA                       | 18.00 | 22.12  | 116.25 |
| CM                       | 16.50 | 29.75  | 144.75 |
| CP                       | 17.00 | 44.00  | 92.25  |
| VA                       | 15.00 | 28.00  | 120.75 |
| VM                       | 16.50 | 27.12  | 96.75  |
| VP                       | 18.00 | 25.50  | 140.25 |

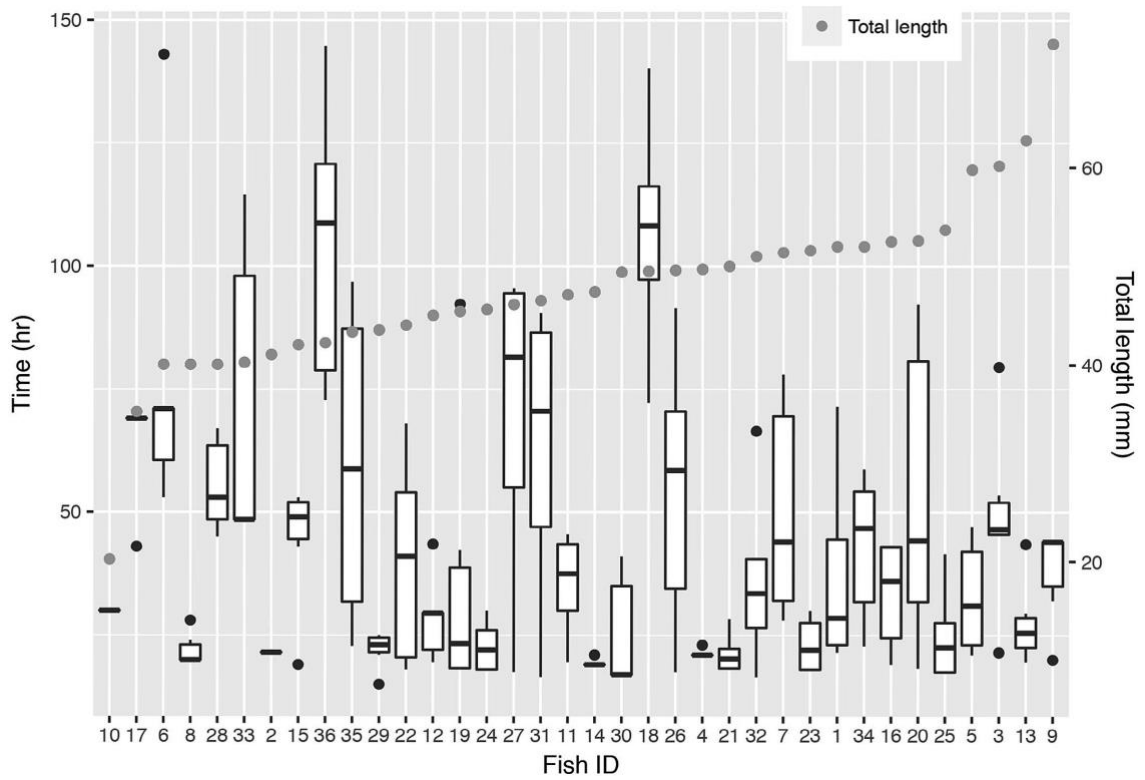
**Table 3.2** Summary of the model coefficients with estimate, standard error along with their 95% confidence intervals, relative t-values, and P-values for each position: dorsal anterior, middle, and posterior (DA, DM, DP); central anterior, middle, and posterior (CA, CM, CP); ventral anterior, middle, and posterior (VA, VM, VP). CA is absent as it is the reference tag position.

| Position     | Estimate | St. error | 2.5% C.I. | 97.5% C.I. | t-value | P-value  |
|--------------|----------|-----------|-----------|------------|---------|----------|
| DA           | 0.504    | 0.139     | 0.236     | 0.773      | 3.620   | 8.46e-04 |
| DM           | 0.284    | 0.131     | 0.032     | 0.538      | 2.168   | 3.64e-02 |
| DP           | 0.090    | 0.125     | -0.150    | 0.330      | 0.725   | 4.73e-01 |
| CM           | 0.092    | 0.138     | -0.173    | 0.358      | 0.671   | 5.06e-01 |
| CP           | 0.045    | 0.139     | -0.222    | 0.315      | 0.326   | 7.46e-01 |
| VA           | 0.060    | 0.139     | -0.207    | 0.329      | 0.432   | 6.68e-01 |
| VM           | -0.005   | 0.126     | -0.247    | 0.238      | -0.041  | 9.68e-01 |
| VP           | 0.064    | 0.132     | -0.189    | 0.318      | 0.486   | 6.29e-01 |
| Total length | -0.011   | 0.009     | -0.028    | 0.007      | -1.198  | 2.38e-01 |



**Figure 3.2** (A) Comparison of attachment time of tags by position (horizontal positions—posterior, middle, and anterior; vertical positions—dorsal, central, and ventral) for left (L) and right (R) side of the fish; box plots show medians, 25th, and 75th percentiles. Black dots represent outliers. (B) Mean attachment time grid by position. Each square represents one of the nine possible tag positions with the relative mean attachment time represented by different color shades (blue-dark purple for mean attachment time between 40–45 hours; light purple-yellow for mean attachment time between 45–50 hours; yellow-orange for mean attachment time between 50–55 hours; orange-red for mean attachment time between 55–60 hours).





**Figure 3.3** Comparison of attachment time (hr) of tags by fish (Fish Identity) ordered by fish size (total length in mm). Box plots show medians, 25th, and 75th percentiles. Black dots represent outliers; gray dots represent total length of fish.

### 3.5 Discussion

Here we developed a new non-invasive method to temporarily tag *Dascyllus aruanus* that is easily detectable both directly by human observers and indirectly by video/photo- cameras.

Firstly, we found that tags lasted longer at specific body positions, namely on the dorsal anterior and dorsal middle locations. This could be due to the fact that fish are more likely to scrape the rounded areas of their body (i.e., central and ventral positions) against the corals while swimming within the tight spaces between the coral branches. Similarly, the motion of the caudal fin could possibly have reduced tag attachment times on the posterior relative to anterior and middle positions of the fish.

Secondly, our results suggest that some individual characteristics other than total fish length were affecting tag life given the variation observed between individual fish (Fig. 3.3). A possible methodological explanation is that we were more or less successful at tagging some fish compared to others, generating inter-individual variability in tag life. This is unlikely to be the case, however, given that only one investigator applied the tags (RB) and all fish were tagged within the same amount of time. Another explanation could relate to variation in behavior among individuals, such as variation in activity or social interactions that may have had an effect on tag attachment times (e.g., Wong et al., 2013).

Importantly, there were no observable negative effects of tagging on the behavior, health, and survival of the fish. Within five minutes (and always within the lifetime of the

tag), the behavior of the marked individuals appeared normal (i.e., regular breathing rate and movements), suggesting that this tagging method can be used for studies that involve behavioral observations. By the end of our experiment, all fish were healthy (i.e., normal activity and regular feeding), and the majority of them possessed new scales as replacements for the ones lost with the tags. In some cases, we observed exposed skin prior to scale replacement, but scale replacement happened within three to four days. Thus, for studies in which it is necessary to temporarily tag the same individuals multiple times, we suggest waiting a reasonable amount of time before marking the fish again using this method (or alternating tag position along the fish's body).

Lastly, one of the interesting features of our new technique is its temporary nature, which may be useful for some types of studies but not others. More specifically, for short-term studies in which it is necessary to observe fish for just a few days, temporary markers may be preferable over permanent ones because they are more cost effective, quick to apply, and naturally wear off over time, hence minimizing the need to permanently alter animals and limiting the negative impacts from trapping and recapturing them to remove tags. Therefore, our new technique could be used widely from ethological studies (e.g., characterizing temporary internal structure of social groups) to ecological studies (e.g., identifying factors influencing movements between different areas or groups). On the other hand, for long-term studies in which the same individuals need to be observed for a prolonged amount of time (e.g., months or years), permanent markers still remain the best choice.

In conclusion, we believe that this new tagging method could be used in future behavioral to ecological studies of this species both in the field and in the lab. Indeed, we were able to use this method, both in the field and in the laboratory, to facilitate a Social Network Analysis study in this species (Branconi et al., unpubl. data). Our study demonstrates the importance of considering tag position rather than just fish body size for the placement of tags to maximize tag attachment. Future work will include the quantification of how behavioral variation between individuals might also influence tag attachment time, the examination of any long-term effects of tagging both in the laboratory and in the field, as well as the comparison of tag attachment times in the laboratory versus field conditions.

The technique could also have different applications in other aquatic organisms (e.g., other fishes and amphibians) in both marine and freshwater environments. We encourage future studies aiming for easy visual detection by observers or video analysis to improve this new non-invasive tagging method, using biodegradable films or other eco-friendly alternatives.

### **3.6 Acknowledgements**

We are particularly grateful to the Boston University MSSP consulting service for their helpful suggestions and comments on the statistical analysis presented in this manuscript. We would also like to thank M. Hing for assistance in the field and LIRS. All work was performed with the approval of the Institutional Animal Care and Use Committee, Boston University (Protocol number: 14-036), the Animal Research Ethics,

University of Wollongong (Protocol number: 14/27), and The Great Barrier Reef Marine Park Authority (Protocol number: G14/ 37263.1). The research was supported by one Warren McLeod Fellowship awarded by Boston University to R. Branconi and one University of Wollongong Target Grant awarded to M. Wong.

## CHAPTER FOUR

### **Comparison of efficiency of direct observations by scuba diver and indirect observations via video camera for measuring reef-fish behavior**

#### **4.1 Abstract**

The present study investigates how the humbug damselfish *Dascyllus aruanus*, subject of a large number of ecological, evolutionary and behavioral studies, responds to the presence of human observers (effect of scuba diver presence-absence) and how the method of data collection (directly by a scuba diver v. indirectly via video camera) may affect the quality of behavioral data. Scuba diver presence had only subtle effects on fish behavior. The efficiency of the method of scoring fish behavior depended on the behavior under consideration: those behaviors that occur in close proximity to the corals were scored more effectively directly by a scuba diver while those that are performed in a more rapid or repetitive fashion were scored more effectively indirectly via video camera. These results provide a foundation for future behavioral research on *D. aruanus* and other fishes where scuba divers or video cameras are the prevalent means of data collection.

## 4.2 Introduction

A central methodological assumption of animal behavior research is that the presence of an investigator and the technique used to collect behavioral data do not alter the behavior of focal animals. Numerous studies have documented the effects of human presence on the distribution, abundance and behavior of different animal species but the majority of animal behavior studies assumes minimal influence of human observers on behaviors of interest. Although the number is small, nearly all studies that have investigated the response of wild and domestic animals to direct human may not truly exist and, ideally, the effect of human presence should be evaluated before interpreting the results of behavioral studies for a wide range of taxa.

When considering aquatic species and environments, the difficulties pertaining to observer effects are even more obvious. In fact, given the necessity for observers to be in close proximity to the studied animals and the visual, olfactory, auditory and somatosensory cues produced by divers, observer presence might be reasonably assumed to influence behavior (Lobel, 2005; Radford et al., 2005). Indeed, the question of whether the presence of divers influences fish behavior, has been previously addressed (Barker et al., 2011; Cubero-Pardo et al., 2011; Di Franco et al., 2013; Nanninga et al., 2017; Smith et al., 2010; Titus et al., 2015). Overall, there is some evidence in the literature demonstrating that divers influence fish behavior (Brignon et al., 2011; Byerly & Bechtol, 2005; Chapman et al., 1974; Dickens et al., 2011; Schmidt & Gassner, 2006; Stanley & Wilson, 1995) but different species respond differently to human presence

(Cole et al., 2007; Dearden et al., 2010; Kulbicki, 1998; Thresher & Gunn, 1986; Watson & Harvey, 2007).




As a practical solution to this issue, underwater video cameras have increasingly been used as an alternative method for collecting behavioral and ecological data (Cappo et al., 2003; Mallet & Pelletier, 2014). However, despite the increasing use of video techniques, it is still not clear whether the data collected directly by scuba divers or indirectly via video cameras are the same, better or worse (Assis et al., 2013; Colton & Swearer, 2010; Longo & Floeter 2012; Lowry et al., 2012; Pelletier et al., 2011; Pereira et al., 2016). Consequently, there is no general consensus regarding whether indirect techniques (such as videos and hydroacoustic data) or direct techniques (such as scuba diver or snorkeler) are more reliable for scoring behavioral data proficiently underwater.

Crucially, while almost all the studies purported to measure the effect of diver presence-absence in addition to the relative efficiency of the different methods being used, most of the experimental designs are confounded. That is to say they compare diver observations without video to video observations without a diver, which means that it is not possible to disentangle whether any observed effects are due to differences in the method of data collection (direct by diver or indirect from video cameras) or differences in diver presence or absence. Ideally, to understand the effect of diver presence, one would therefore compare indirect video observations with a diver present and a diver absent; similarly, to understand the effect of alternative methods of data collection, one would compare direct diver observations to indirect video observations taken



simultaneously (Table 4.1). More studies are evidently needed to disentangle the diver presence–absence effects from the effects due to the different methods used for measuring fish behavior.

**Table 4.1** Schematic of the experimental procedure showing how the data from each group were collected in three different ways: indirectly with video camera and with scuba diver absent; indirectly with video camera and with a scuba diver present; directly by scuba diver observations and with video camera present. For each treatment, the focal methods of data collection are highlighted in black

| TREATMENT   | COMPARISON  | COMPARISON   |
|---|---|--|
|  <p><b>VIDEO SCORING - SCUBA DIVER ABSENT</b></p>    | <p>Compare the data collected from the two video recordings to determine the effect of scuba diver presence versus absence on fish behaviour (n=20)</p> |  |
|  <p><b>VIDEO SCORING - SCUBA DIVER PRESENT</b></p> |   | <p>Compare data collected directly by the diver to data collected indirectly from the video to determine the efficiency of different methods of data collection (n=20)</p> |
|  <p><b>SCUBA DIVER SCORING - VIDEO PRESENT</b></p> |   |  |

The overall goals of this study were to assess whether the presence of a human observer influenced behavior and compare alternative methods of measuring behavior using the humbug damselfish, *Dascyllus aruanus* (L. 1758). *Dascyllus aruanus* has already been the focus of extensive behavioral and ecological research (more than 100 published papers), making this species an ideal model. *Dascyllus aruanus* is widespread

throughout the Indo-Pacific region, living in social groups in close association with colonies of certain branching corals (Holbrock et al., 2000; Sale, 1971). Within each discrete coral patch there is a single group, with a range size between 2–10 individuals (Holbrock et al., 2000; Sale 1972), of mostly non-relatives (Buston et al., 2009; Sale, 1971). Each group is composed of males, females and non-breeders (Cole, 2002; Wong et al., 2012), with the mating system shifting from monogamy to polygyny to polygynandry and the number of non-breeders increasing, with increasing group size (Fricke; 1980; Wong et al., 2012).

Here, an ethogram describing *D. aruanus* behavior was developed and two hypotheses were tested: 1) the presence of a scuba diver would influence fish behavior and 2) there would be a difference in the efficiency of collecting data directly by scuba diver or indirectly from video cameras that depended on the behavior being scored. More generally, the present study developed a framework for determining the effect of observer presence and data collection method on fish behavior, that could be widely applied in other studies and taxa.

### 4.3 Methods

#### 4.3.1 Study population

The population of *D. aruanus* in Lizard Island Lagoon on the northern Great Barrier Reef, Australia (14°40' S, 145° 27' E) was studied from November to December 2014. Two dive sites were selected: Bird Islet (14° 41.271'S, 145° 27.655' E) and Palfrey island (14°41.764' S, 145° 26.890' E). Within the dive sites, the reef consisted of areas of rubble and living corals interspersed with extensive areas of sand.

#### 4.3.2 Development of ethogram (metrics of behavior)

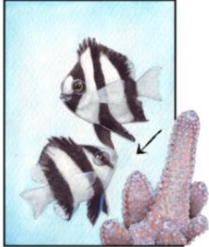



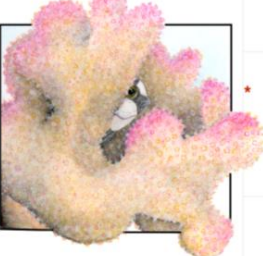
First, an ethogram was developed to determine and quantify the behavioral repertoire of *D. aruanus*. At each site, six corals occupied by a single group of *D. aruanus* were randomly selected and each fish of each group was observed for 10 min by a scuba diver and all their behaviors directly scored. Thirty-six different behaviors were identified in total, which were then classified into five main categories of behavior: aggressive, social, maintenance, reproductive and submissive behaviors (Table 4.2).

Although subjective, the different behaviors were assigned to categories following previous work in which standard ethograms have been developed to score fish behavior effectively (Matthews and Wong, 2015; Sopinka et al., 2009) as follows: (a) aggressive if the behavior of the focal fish resulted in either contact (e.g., bite or ram) or non-contact aggression (e.g., chasing, aggressive display); (b) social if the behavior of the focal fish involved either non-aggressive contact (e.g., soft touch; heterospecific affiliation) or

non-contact (e.g., meeting; following); (c) maintenance if the behavior of the focal fish resulted in the acquisition of different resources such as food (e.g., feeding in the water column, feeding from the bottom or from the coral), shells or rocks (e.g., carrying) or shelter (e.g., retreat; dock), or appeared to serve some other bodily maintenance function (e.g., shaking, scratching-scraping, yawning and flexion); (d) reproductive if the behavior of the focal fish was related to spawning (e.g., leading to spawn, pseudo spawning) or nest-egg care (e.g., mouthing; fanning; nest preparation; nest control); and (e) submissive if the behavior of the focal fish was a response to the aggressive behaviors performed by another member of its own group (e.g., flee, hiding, head down, submissive display). Two behaviors (signal jumping and fins rising) were assigned to multiple categories (social, maintenance and reproductive) because they occurred in multiple contexts.

For a comprehensive analysis of these behaviors, we present analyses of both the five categories and the individual behaviors. All 36 different behaviors presented in the ethogram were included into the statistical analysis of the five main categories of behavior. Nineteen of the 36 different behaviors were used for the statistical analysis of individual behaviors due to the very low occurrence of the other 17 behaviors (Table 4.2).

**Table 4.2** Ethogram for the humbug damselfish *Dascyllus aruanus*. Thirty-six different behaviors were classified into five main categories: aggressive, social, maintenance, reproductive and submissive. All 36 different behaviors presented in the ethogram were included into the analysis of the five main categories; only 19 of the 36 different behaviors (indicated by a star) were included in the analysis of individual behaviors. The behaviors assigned to multiple categories are highlighted in grey.

|                                    | CATEGORY   | BEHAVIOUR                                     | DESCRIPTION   |                |   |
|------------------------------------|--|---|---|----------------|---|
| AGGRESSIVE                         |   | * CHASING                                     | Focal fish quickly swims towards another fish (short chase : long chase : chase-chase : focal fish quickly darts towards another fish then chases it in turn; -hook - focal fish swims towards another fish then turns away (usually displayed by subordinate fish to a fish higher in the hierarchy).  |                |   |
|                                    |  | * CHASING HETERO SPECIFICS                    | see description above   |                |   |
|                                    |  | BITING  | Focal fish bites another fish   |                |   |
|                                    |  | BITING HETERO SPECIFICS                       | see description above   |                |   |
|                                    |  | * AGGRESSIVE DISPLAY<br>(in the figure)       | Focal fish raise all fins and swim upwards to another fish and then takes an oblique position and quivers and finally swims in parallel   |                |   |
|                                    |  | * AGGRESSIVE DISPLAY AGAINST HETERO SPECIFICS | see description above   |                |   |
|                                    |  | RAM   | Focal fish makes contact with another fish using the head or mouth region, but no obvious bite is taken (jaws remain closed)  |                |   |
| SOCIAL                             |    | * SIGNAL JUMPING<br>(in the figure)           | The signal jump consists of a fish rising in the water column and then rapidly swimming down while making a sound. Throughout the signal jump the first black bar of the focal fish has a grey coloration   |                |   |
|                                    |  | * FOLLOWING                                   | Focal fish follows another one within the group   |                |   |
|                                    |  | * FINS RISING                                 | Focal fish raise all fins   |                |   |
|                                    |  | MEETINGS                                      | Focal fish meets another one and swim together  |                |   |
|                                    |  | SOFT TOUCH                                    | Focal fish nips or softly makes contact with another individual   |                |   |
|                                    |  | HETERO SPECIFIC AFFILIATION                   | Focal fish receives cleaning services by a cleaner fish   |                |   |
| MAINTENANCE                        |   | * FEEDING IN THE WATER COLUMN                 | Focal fish orients towards food and opens its mouth to take in particles in the water column  |                |   |
|                                    |  | * FEEDING FROM THE CORAL                      | Focal fish orients towards food and opens its mouth to take in particles from the coral's surface   |                |   |
|                                    |  | * FEEDING FROM THE BOTTOM                     | Focal fish orients towards food and opens its mouth to take in particles from the bottom  |                |   |
|                                    |  | * DARTING                                     | A spontaneous, quick and short swim with no obvious direction (not as a response to particular evident stimuli)   |                |   |
|                                    |  | * SHAKING                                     | Focal fish quivers; the whole body trembles   |                |   |
|                                    |  | * SCRATCHING-SCRAPING                         | Focal fish scrapes very quickly its side or underside against a surface   |                |   |
|                                    |  | * POINTING-FREEZING                           | Focal fish is suddenly immobilized (not as a response to particular evident stimuli)  |                |   |
|                                    |  | * RETREAT                                     | Focal fish quickly swims inside coral branches or holes because of external stimuli ( but not as a response to received aggressive behaviours)  |                |   |
|                                    |  | * YAWNING AND FLEXION<br>(in the figure)      | State of maximal stretch of the body musculature: the focal fish sank slightly in the water in a head-up position with the mouth opened and the pinnacules projected forward to their maximal extent, all the fins are raised maximally ( the fish spreads the caudal fin and the pelvic fins and momentarily stopped the sculling motion of the pectoral ones) |                |   |
|                                    |  | * DOCK  | Focal fish abruptly swims quickly in the direction of the coral without obvious external stimuli  |                |   |
|                                    |  | REPRODUCTIVE                                  |    | SIGNAL JUMPING | The signal jump consists of a fish rising in the water column and then rapidly swimming down while making a sound. Throughout the signal jump the first black bar of the focal fish has a grey coloration |
|                                    |  |   |   | FINS RISING    | Focal fish raise all fins   |
| CARRYING                           | Focal fish takes in a mouthful of something (e.g. a rock or a shell) and swims to a different area before spitting it out  |   |   |                |   |
| LEADING TO SPAWN                   | Focal fish, after performing a signal jumping to attract a female, is followed by the female to the nest   |   |   |                |   |
| FANNING                            | Fanning the eggs with the caudal fin   |   |   |                |   |
| MOUTHING                           | Focal fish peck at the eggs with its mouth   |   |   |                |   |
| NEST PREPARING                     | Focal fish cleans the area of the nest by actively removing algae with its mouth, by carrying away little rocks or shells and quivers its body against the substratum displacing part of it  |   |   |                |   |
| * NEST CONTROLLING                 | Focal fish checks the condition of the nest going repetitively outside and inside it   |   |   |                |   |
| REPRODUCTIVE SHAKING-SCRAPING      | Focal fish quivers right next to another fish; the whole body trembles   |   |   |                |   |
| PSEUDO SPAWNING<br>(in the figure) | Two focal fish move in little circles following and touching each other while their whole bodies tremble; this behaviour usually starts in the water column and continues repetitively in the direction of the nest where they repeat these movements multiple times, but against a hard surface simulating a spawning behaviour |   |   |                |   |
| SUBMISSIVE                         |   | SIGNAL JUMPING                                | The signal jump consists of a fish rising in the water column and then rapidly swimming down while making a sound. Throughout the signal jump the first black bar of the focal fish has a grey coloration. During courtship behavior, the focal fish performs a signal jump to attract the female and then leads her to spawn                                   |                |   |
|                                    |  | FLEE  | Focal fish quickly swims away from another fish as a response to received aggressive behaviours   |                |   |
|                                    |  | * HIDING<br>(in the figure)                   | Focal fish quickly swims inside coral branches or holes into the rocks to place itself out of another fish's view as a response to received aggressive behaviours   |                |   |
|                                    |  | HEAD DOWN                                     | The head of the focal fish is directed downwards (sometimes entirely vertical) and the tail is upwards  |                |   |
| SUBMISSIVE DISPLAY                 | The head of the focal fish is directed downwards (sometimes entirely vertical), the tail is upwards and the whole body trembles  |   |   |                |   |

### 4.3.3 Experimental procedure and data collection

At each site, 12 corals occupied by a single group of *D. aruanus* were located and mapped. These groups were situated at a distance of at least 8 m from each other and there was no movement of individuals between groups. The species and the size of each coral were recorded. Coral size was determined using a measuring tape by taking the length, the width and the height of the coral colonies and calculating the ecological volume in  $\text{cm}^3$  (Shaish et al., 2007). Group sizes, which ranged from 3 to 7 fish, were determined by counting the number of resident *D. aruanus* in the focal host coral. All work was conducted using snorkel and scuba.

To investigate the effect of scuba diver presence or absence on multiple categories of fish behavior, paired video recordings of each group ( $n = 20$ ) were conducted (Table 4.1). Behavioral data obtained from the video recordings of the same group taken the same day with and without the scuba diver present were compared by the same observer. Using GoPro Hero 3 ([www.gopro.com](http://www.gopro.com)) cameras fixed on tripods, each group was recorded twice between 10:00 and 16:00 hours. The camera was placed at 3 m from the colonies and the scuba diver was positioned on one side of the camera at 4 m from the colonies. The order of the paired recordings was randomized and recordings were made in quick succession, one after the other. Within each group, the behaviors of the fish were scored sequentially: each fish of each group was observed for 10 min and all the behaviors were indirectly scored by one observer (R.B). Both the video recordings and the behavioral measurements commenced 5 min after the scuba diver's arrival and setting

up the tripod and camera, to allow acclimation of the fish. All the videos were examined using the program VLC Media player 2.1.3 ([www.vlc-media-player.en.uptodown.com](http://www.vlc-media-player.en.uptodown.com)) and the resulting behavioral data were analyzed using mixed model analysis.

To investigate the effectiveness of alternative methods of scoring multiple categories of fish behavior, behavioral data obtained from direct measurements by the scuba diver and behavioral data obtained indirectly from the video recordings of the same group taken at the same time were compared ( $n = 20$ ) (Table 4.1). Within each group, the behaviors of the fish were scored sequentially. It was possible to follow one individual at a time directly and indirectly (each fish of each group was observed for 10 min). It was not possible, however, to identify the same individual across direct and indirect observations as there was no relevant apparent phenotypic variation between fish, and standard methods of tagging were unsuitable for this species due to their specific ecology and morphology or did not result in marks that were visible from a distance and via video recordings. Behavioral data were analyzed using mixed model analysis.

#### *3.3.4 Statistical analysis*

To determine whether counts of behavior differed based on scuba diver presence or absence and on method of data collection (directly by scuba diver or indirectly from video), generalized linear models (GLM) were used in SPSS 24 (IBM; [www.ibm.com](http://www.ibm.com)). The dependent variable was the frequency of each fish behavior (19 different behaviors and five different categories of behavior). The analyses adjusted for multiple comparisons using least significant differences method. Two hypotheses were tested: that there would

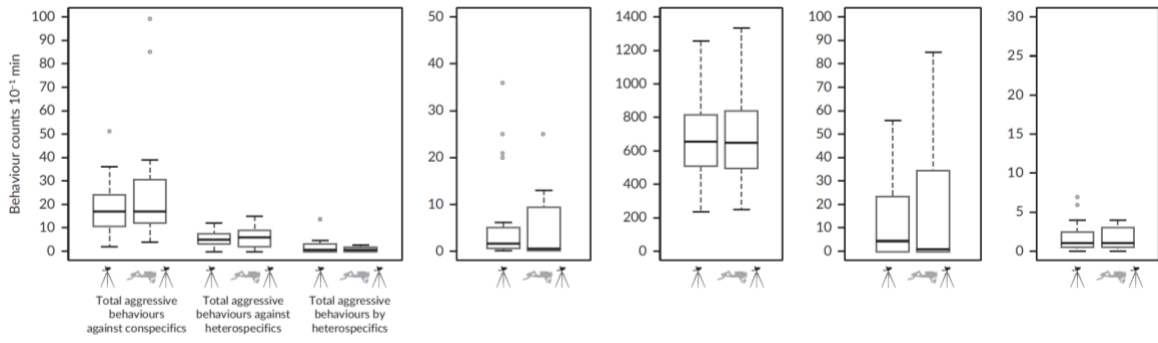
be differences in the counts of behavior scored when the diver is present v. absent, in which case the primary independent variable was “diver present” v. “diver absent” (categorical); that there would be differences in the counts of behavior scored directly by the scuba diver v. indirectly from the video cameras, in which case the primary independent variable was “video observations” v. “diver observations” (categorical). Group size, coral size, coral species (fixed effects) were included in the models to control for their potential effects on behavior. Group identification (ID; random effect) was also included in the models to control for the lack of independence between multiple measures from the same group.

## 4.4 Results

### 4.4.1 *The effect of scuba diver presence–absence on fish behavior*

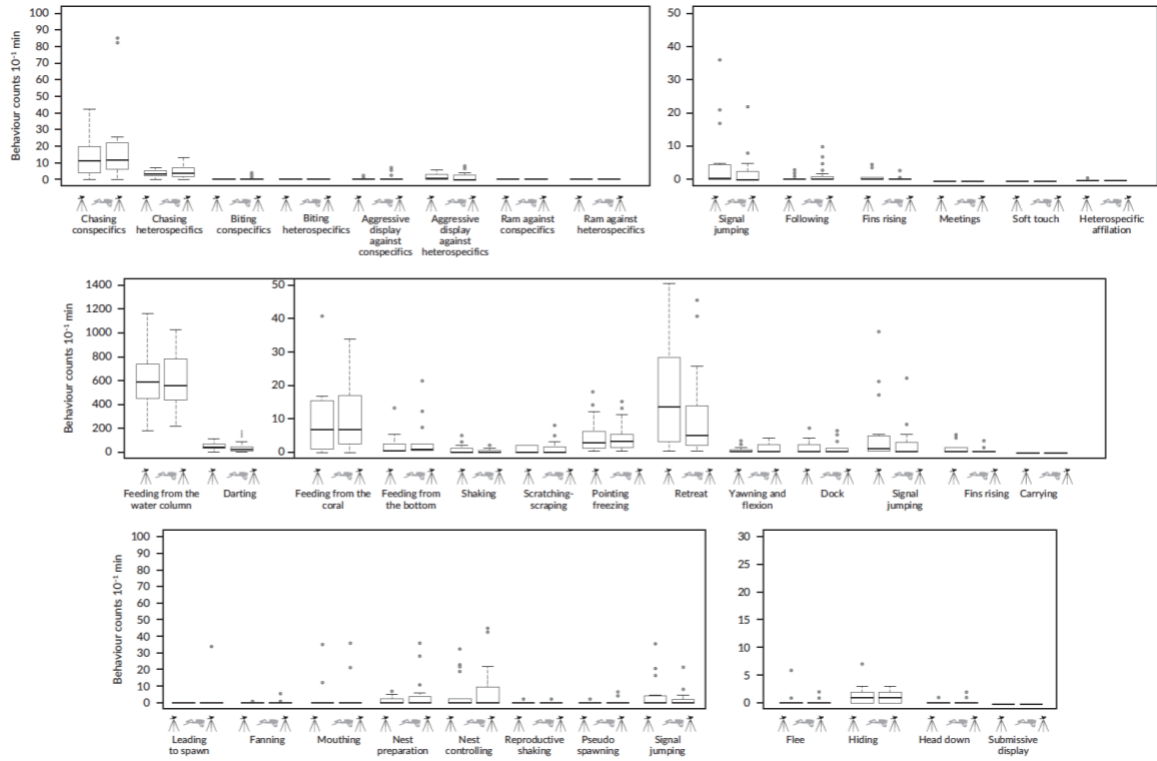
The behavior of *D. aruanus* was only mildly affected by the scuba diver’s presence (Figure 4.1). Considering the 5 major categories of behavior, there were no significant differences in the number of aggressive, social, maintenance and submissive behaviors scored from video when the scuba diver was present v. when the scuba diver was absent (GLM, all  $P > 0.05$ ; Figure 4.1). However, there was a significant difference in the number of reproductive behaviors between the two treatments (GLM,  $F_{1,28} = 4.357$ ,  $P < 0.05$ ; Figure 4.1), with the number of reproductive behaviors being slightly higher when the scuba diver was present (mean = 0.180) than when the scuba diver was absent (mean = 0.122).





**Figure 4.1** Box-plot (—, median; □, 25th and 75th percentiles; ▮, 95% range; ●, outliers) comparison of diver presence-absence on five major classes of behavior in *Dascyllus aruanus*: aggressive, social, maintenance, reproductive, submissive. The focal method of data collection is highlighted in black.

Of the 19 specific behaviors, the number of feeding in the water column events was significantly lower (GLM,  $F_{1,28} = 4.230$ ,  $P < 0.05$ ) and the number of yawning and flexion events was significantly higher (GLM,  $F_{1,28} = 4.230$ ,  $P < 0.05$ ) when the scuba diver was present than when the scuba diver was absent. However, there were no significant differences in the remaining 17 of 19 specific behaviors counted from video when there was a scuba diver present v. when the diver was absent (GLM, all  $P > 0.05$ ; Figure 4.2).



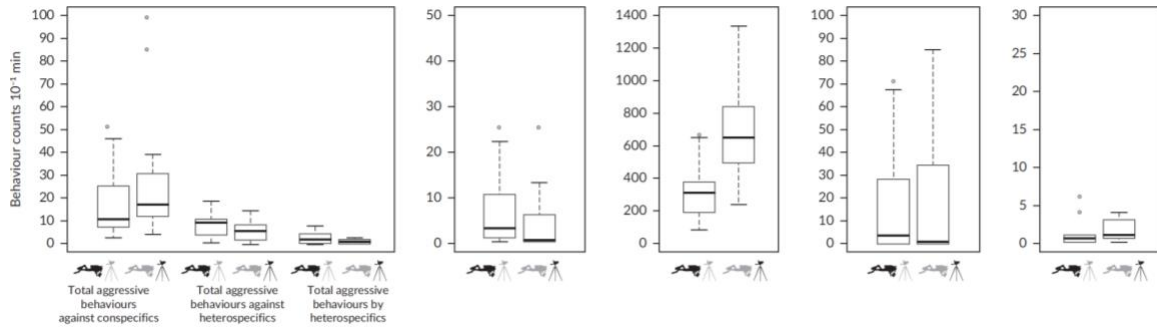
**Figure 4.2** Box-plot (—, median; □, 25th and 75th percentiles; ▮, 95% range; ●, outliers) comparison of diver presence–absence for all 36 behaviors of *Dascyllus aruanus* within five classes: aggressive, social, maintenance, reproductive, submissive. The focal method of data collection is highlighted in black.

It is worth noting that there were significant effects of group size on 4 out of 5 categories of behaviors: aggressive behaviors (total aggressive behaviors against conspecifics, GLM,  $F_{4,3} = 23.528$ ,  $P < 0.05$ ; total aggressive behaviors by heterospecifics, GLM,  $F_{4,28} = 5.339$ ,  $P < 0.01$ ); social behaviors (GLM,  $F_{4,4} = 15.390$ ,  $P < 0.05$ ); maintenance behaviors (GLM,  $F_{4,9} = 22.552$ ,  $P < 0.001$ ); reproductive behaviors (GLM,  $F_{4,28} = 247.616$ ,  $P < 0.001$ ). Also, there were significant effects of group size in 17 out of 19 different behaviors.

Similarly, there were significant effects of coral species in 4 out of 5 categories of behaviors: aggressive behaviors (total aggressive behaviors against heterospecifics, GLM,  $F_{5,28} = 7.844$ ,  $P < 0.001$ ; total aggressive behaviors by heterospecifics, GLM,  $F_{5,28} = 78.012$ ,  $P < 0.001$ ); maintenance behaviors (GLM,  $F_{5,28} = 13.955$ ,  $P < 0.001$ ); reproductive behaviors (GLM,  $F_{5,28} = 5530.843$ ,  $P < 0.001$ ); submissive behaviors (GLM,  $F_{5,28} = 9.645$ ,  $P < 0.001$ ). Also, there were significant effects of coral species in 17 out of 19 different behaviors. Finally, there were significant effects of coral size in 4 out of 19 different behaviors.

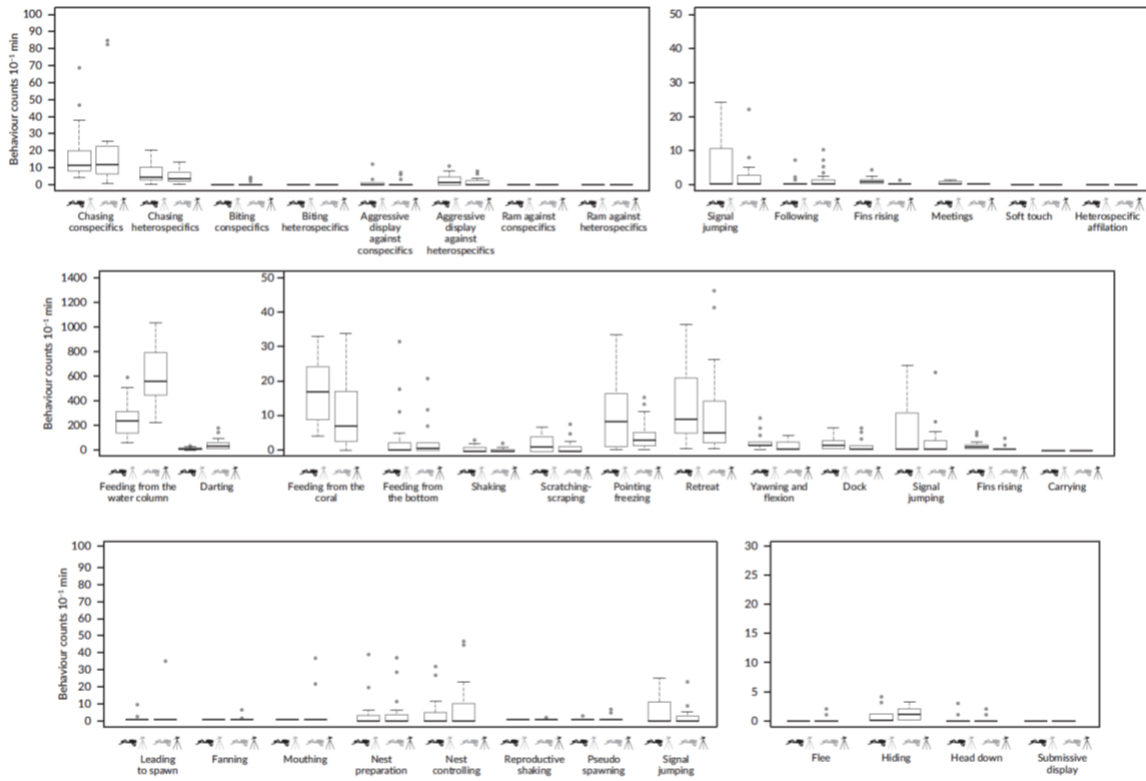
#### *4.4.2 The effect of method of scoring fish behaviors directly by scuba diver v. Indirectly from video cameras*

The efficiencies of the two techniques for scoring behavior were different (Figure 4.3). Considering the 5 major categories of behavior, there were significant differences in the number of total aggressive behaviors by heterospecifics (GLM,  $F_{1,28} = 12.451$ ,  $P < 0.001$ ), social (GLM,  $F_{1,28} = 4.670$ ,  $P < 0.05$ ) and maintenance (GLM,  $F_{1,28} = 220.645$ ,  $P < 0.001$ ) behaviors scored directly by a scuba diver and taken indirectly from video cameras (Figure 4.3). Specifically, maintenance behaviors were significantly lower when directly scored by a scuba diver, whereas total aggressive behaviors by heterospecifics and social behaviors were significantly higher when scored directly by a scuba diver than indirectly from video cameras (Figure 4.3).



**Figure 4.3** Box-plot (—, median; □, 25th and 75th percentiles; ▮, 95% range; ●, outliers) comparison of video and direct diver observations on five major classes of behavior in *Dascyllus aruanus*: aggressive, social, maintenance, reproductive, submissive. The focal method of data collection is highlighted in black.

Of the 19 specific behaviors, four behaviors showed lower counts when directly scored by scuba diver compared with indirectly scored from video cameras, namely feeding in the water column (GLM,  $F_{1,28} = 80.262$ ,  $P < 0.001$ ), nest controlling (GLM,  $F_{1,2} = 38.118$ ,  $P < 0.05$ ), darting (GLM,  $F_{1,28} = 85.123$ ,  $P < 0.001$ ) and hiding (GLM,  $F_{1,28} = 4.265$ ,  $P < 0.05$ ), while one behavior, feeding from the coral (GLM,  $F_{1,28} = 25.315$ ,  $P < 0.001$ ) registered higher counts when scored directly by a scuba diver. However, there were no significant differences in the remaining 14 out of 19 specific behaviors between direct by scuba diver or indirect from video cameras (all  $P > 0.05$ ; Figure 4.4).



**Figure 4.4** Box-plot (—, median; □, 25th and 75th percentiles; ▭, 95% range; ●, outliers) comparison of video and direct diver observations on all 36 *Dascyllus aruanus* behaviors within five classes: aggressive, social, maintenance, reproductive, submissive. The focal method of data collection is highlighted in black.

It is worth noting that there were significant effects of group size in 2 out of 5 categories: aggressive behaviors (total aggressive behaviors v. conspecifics, GLM,  $F_{4,28} = 3.451$ ,  $P < 0.05$ ); maintenance behaviors (GLM,  $F_{4,17} = 72.103$ ,  $P < 0.001$ ). Also, there were significant effects of group size in 11 out of 19 different behaviors. Similarly, there were significant effects of coral species in 3 out of 5 categories: aggressive behaviors (total aggressive behaviors against conspecifics, GLM,  $F_{5,28} = 4.911$ ,  $P$  value  $< 0.001$ ; total aggressive behaviors by heterospecifics, GLM,  $F_{5,28} = 876.008$ ,  $P <$

0.001); social behaviors (GLM,  $F_{5,28} = 131.366$ ,  $P < 0.001$ ); maintenance behaviors (GLM,  $F_{5,28} = 4.472$ ,  $P < 0.01$ ). Also, there were significant effects of coral species on 14 out of 19 different behaviors. Finally, there were significant effects of coral size in 2 out of 19 different behaviors.

## 4.5 Discussion

This study showed that scuba diver presence had only subtle effects on the behavior of *D. aruanus* and that the efficiency of the method of scoring the behavior of *D. aruanus* depended on the behavior under consideration.

### 4.5.1 *The effect of scuba diver presence-absence on fish behavior*

Our findings indicate that the presence of stationary scuba divers had a relatively minor influence on the behavior of *D. aruanus*. Four out of the five major categories of behavior, namely aggressive, social, maintenance and submissive did not show any significant differences in occurrence when there was a scuba diver present v. when absent. Surprisingly, however, there were more reproductive behaviors scored when a diver was present. Currently, there are no definitive explanations for this outcome. However, it is plausible that *D. aruanus* may perceive the presence of a scuba diver as a threat (Frid & Dill, 2002). In response to perceived threats, individuals have been shown to modulate their reproductive behaviors in counterintuitive ways, as both the optimal investment in current and future reproduction and the level of predation risk differ

between species and between individuals within the same species (Franklin et al., 2014; Magnhagen, 1990). Future studies are needed to clarify this matter.

Focusing on the specific behaviors, feeding in the water column was significantly lower and yawning and flexion were significantly higher when the scuba diver was present v. absent. Once again, it's plausible that both results are due to the fish perceiving the scuba diver as a threat. Considering feeding in the water column, this could be less common in the presence of the diver because one key behavioral responses of some damselfish to perceived predation risk is adjusting the distance at which they hover away from their host colony (Gauff et al., 2018). Turning to consider yawning and flexion behavior, this could be more common in the presence of the diver because, according to the theory of causality and function of yawning (Rasa, 1971), the performance of yawning and flexion in fishes is a mechanism to increase muscle tonus and aid in preparing the animal for action, i.e., fleeing from the perceived threat.

#### *4.5.2 The effect of method of scoring fish behaviors directly by scuba diver v. indirectly from video cameras*

The relative efficiency of each technique, in terms of the number of multiple categories and specific fish behaviors collected, depended on the behavior being scored. Considering the five major categories of behavior, there were significant differences in the number of maintenance behaviors, which were lower when directly scored by a scuba diver than indirectly from video cameras and total aggressive behaviors by heterospecifics and social behaviors, which were higher. A possible explanation for these

results relies on the evaluation of the specific behaviors analyzed. Specifically, those behaviors that occur in close proximity to the coral (e.g., chasing heterospecifics, aggressive display against heterospecifics, feeding from the coral and signal jumping) are scored more effectively directly by a scuba diver because of the better visibility and the possibility of varying the view- point that is not possible from video cameras. Conversely, for behaviors that are performed in a more rapid or repetitive fashion (e.g., feeding in the water column, nest controlling, darting and hiding) indirect video camera measurements are more effective because of the opportunity of viewing the same captures at different speeds and multiple times.

In conclusion, it is fundamental for animal behavior studies involving direct human observations to investigate the effect of researcher presence. Ideally, there would be no effect of observer presence, but this will not be the case for all experimental systems. Fortunately, observer effects may not affect the conclusions of studies too greatly, as long as observer presence does not interact with the treatments of interest; i.e., when observer presence biases behavior in the same way across all treatments or all behaviors. However, there is some evidence in the literature demonstrating that different species respond differently to human presence (Cubero-Pardo et al., 2011; Dearden et al., 2010; Kulbicki, 1998; Thresher & Gunn, 1986; Watson & Harvey, 2007) and human presentation style; e.g., scuba divers, free divers and snorkelers or rebreather and open-circuit scuba divers (Cole et al., 2007, Dearden et al., 2010). Thus, the observer effect should be carefully evaluated on a case-by-case basis to avoid collections of biased data, false conclusions and misconceptions about the behavior of the species studied.



Most of the previous assessments of the effect of scuba diver presence–absence have been confounded (i.e., comparing diver observations with video absent to video observations with a diver absent), so that it is not possible to determine whether any observed effects are due to differences in the method of data collection or differences in diver presence-absence itself. Here, we present an alternative experimental design that allows us to assess the effect of diver presence absence and the efficiency of the method of data collection without them being confounded. Our results suggest that, while the overall frequency of most behaviors of *D. aruanus* was not intensely affected by the presence of a scuba diver, there were subtle influences on some behaviors that may be important depending on the questions being addressed. Further, our results show that one method of data collection (direct by a scuba diver or indirect via video) could be preferable over the other, depending on the species and on the behavior of interest. This experimental design presented here could be adopted as an observer assay to indicate the extent of observer influence in any animal system, which will be a useful addition at the outset of any behavioral study.

#### **4.6 Acknowledgements**

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## CHAPTER FIVE

### **Ecological constraints influence cooperation and conflict in the humbug damselfish**

#### *Dascyllus aruanus*

##### **5.1 Abstract**

Animal societies vary greatly in levels of cooperation and conflict. Explaining the causes of this variation is a major goal of evolutionary biology. As with other societal features, relatedness, competitiveness, and ecological constraints have been identified as key factors explaining variation in cooperation and conflict. However, the exact mechanisms influencing such variation remains still unclear. Coral reef fishes offer us the possibility to conduct manipulative experiments that aren't necessarily possible in other social vertebrates potentially helping us better understanding the causes and mechanism of this variation. The humbug damselfish *Dascyllus aruanus* lives in social groups that occupy coral heads where ecological constraints seem relevant and there is naturally occurring variation in cooperation and conflict among groups. Here, we use *D. aruanus* to test experimentally the hypotheses that the frequency of cooperative and conflictive interactions will be influenced by a variation in ecological constraints in the form of habitat availability surrounding groups. We manipulated ecological constraints by providing a vacant coral near to social groups. We examined the effect of relaxing ecological constraints in this way on the frequency of cooperative and conflictive interactions between individuals, and we examined how the effect was modulated by natural variation in individual traits, social context, and ecological context. We found that relaxed ecological constraints result in increased conflict and decreased cooperation in

some individuals, some social contexts, and some ecological contexts. These findings are consistent with the idea that individuals will be more inclined to compete with each other and less inclined to cooperate with each other as their outside-group options improve. This provides new insights into the underlying causes of cooperation and conflict in animal societies.

## 5.2 Introduction

Understanding the causes of inter- and intraspecific variation in animal societies is a major goal of evolutionary biology. Societies vary greatly in their reproductive partitioning, levels of cooperation and conflict, group size, structure, and stability. It is widely appreciated that reproductive partitioning and group stability are dependent on a combination of genetic, social, and ecological factors (Hamilton, 1964; Emlen, 1982; Keller & Reeve 1994). When relatedness is high, there are strong competitive asymmetries, and there are constraints on independent breeding, subordinate individuals are more likely to forgo their own reproduction and remain within a group (e.g., Pruett-Jones & Lewis, 1990; Walters et al, 1992; Heg et al., 2004; Bergmüller et al., 2005; Halliwell et al., 2017; Brouwer & Cockburn 2020). These same factors - relatedness, competitiveness, and ecological constraints - are likely to influence levels of cooperation and conflict within groups also, but exactly how and why they do so in nature remains unresolved. Here, we focus on the role of ecological constraints in determining levels of cooperation and conflict in social groups.

Several factors can be encompassed by the umbrella term “ecological constraints”: high habitat saturation or limited habitat availability (i.e. no high-quality habitat vacancies), high interspecific or intraspecific competition (i.e. spatially limited resources surrounding individuals), low habitat stability (i.e. temporally limited resources surrounding individuals) and high risk of movement and predation (i.e. is dangerous to move between patches of suitable habitat due to high risk of predation) are just some examples of ecological constraints. Interestingly, the relaxation of these factors, both natural or experimentally induced, (e.g. a decrease in individuals’ risk of movement due to reduced predator density; a decrease in habitat saturation due to individual dispersal/removal or by the occurrence of new habitat vacancies) have the potential to influence individuals’ costs and benefits of living within a social group and, therefore, affect within group cooperation, conflict and overall group stability.

Multiple models have been created to predict how variations in ecological constraints, influence cooperation and conflict within social groups. The ecological constraints hypothesis (Emlen; 1982) suggests that conflictive interactions will be more likely when there are relaxed ecological constraints, and cooperative behaviors will be more likely when there are harsh ecological constraints (Emlen, 1982). Transactional models of reproductive skew, in which group members yield shares of reproduction to each other in return of cooperation, similarly, predict that groups will be more prone to conflict when there are relaxed ecological constraints (Johnstone, 2000; Reeve, 2000; Cant & Johnstone 2009; Buston & Zink 2009). On the other hand, tug-of-war models of

reproductive skew, in which group members invest in group resources in a tug-of-war over their respective reproductive shares, suggest that i) the degree of within group conflict will increase as ecological constraints on solitary breeding increase (i.e. the noncooperative options become less attractive) and that ii) decreasing subordinates' relative benefits of cooperation will result in a decrease of dominant's aggression toward subordinates (Reeve et al. 1998; Reeve & Shen 2006). Furthermore, a model that synthesizes the ecological constraints hypothesis, the life-history hypothesis and the benefits-of-philopatry hypothesis suggests that when there is a high likelihood of territory inheritance, which is generally promoted by harsh ecological constraints, there will be greater cooperation within groups (Pen & Weissing 2000). Lastly, the dual benefits framework model suggests that cooperation will be promoted, and driven by resource defence benefits, in social groups where ecological constraints limit independent breeding (Shen et al. 2017).

Few studies have tested how ecological constraints affect cooperation and conflict in animal groups. Shen et al. (2012), for example, demonstrated that high rainfall conditions (i.e., harsher ecological constraints), which have negative influence on individual thermoregulation, foraging and nesting efficiency, reduce social conflict and promote cooperative strategies in the joint-nesting Taiwan yuhinas *Yuhina brunneiceps*, resulting in better fitness outcomes for individuals. Marshalls et al. (2016), using the cooperatively breeding banded mongoose *Mungos mungo*, showed that harsher ecological constraints, in the form of more variable ecological conditions (i.e. changes in rainfall),

determined increased cooperative efforts by males, as a result of poorer female survival and male-biased group compositions. Furthermore, Chen et al. (2020) demonstrated that in the facultative cooperatively breeding Asian burying beetle *Nicrophorus nepalensis*, interspecific competition (i.e., harsher ecological constraints) promotes the transition from intraspecific conflict to cooperation. Therefore, these studies showed that natural variation in ecological constraints can influence the amount of social conflict and cooperative efforts between individuals. In addition, few experimental studies conducted on terrestrial or freshwater social vertebrates have investigated the effect of manipulating ecological constraints on cooperation and conflict within groups (Bergmüller et al. 2005; Heg & Taborsky, 2010; Tibbetts & Reeve, 2008; Lindstedt et al., 2018; Liu et al.; 2020); in general, these studies showed that also the experimental manipulations of ecological constraints can modulate the frequency of cooperative and conflictive interactions between individuals. Interestingly, some coral reef fish that form stable social groups composed of non-relatives (e.g. damselfish, gobies, cardinalfish), offers us the possibility to conduct manipulative experiments on ecological constraints that aren't necessarily possible in other social vertebrates, and allows us to investigate the causes and mechanisms of the variation in cooperation and conflict within animal groups from a slightly different perspective (Rueger et al 2021a).

In the last 20 years, a growing number of studies suggested that marine fishes are good models to investigate the balance between cooperation and conflict within social groups (Rueger et al 2021a). Some coral reef fishes live in social groups that present a striking resemblance to the societies of cooperatively breeding mammals, birds,

freshwater fishes and invertebrates (Taborsky & Limberger, 1981; Emlen, 1991; Sherman et al., 1995; Duffy et al., 2000; Buston, 2002; Wong, 2007; Bourke, 2011). Interestingly, multiple cross-factored experiments showed that ecological constraints, in the form of habitat saturation and risks of movement, are a driving force behind the formation of social groups in coral reef fishes (Wong, 2010; Branconi et al., 2020). In addition, in different coral reef fishes, the amount of social interactions between group members has been shown to depend also on individuals' size and dominance rank (Fricke & Fricke 1977; Colleter & Brown, 2011; Dey et al. 2012; Rueger et al 2021b). This suggests that social context is another driving evolutionary force for the formation and maintenance of coral reef fish societies (e.g., Buston & Cant 2006; Wong et al. 2007; Branconi et al., 2020). However, most of these studies have been conducted in species with relatively inflexible social systems, low/no individual mobility, and low/no variation in reproductive skew, e.g., the clown anemonefish *Amphiprion percula*; the emerald coral goby *Paragobiodon xanthosoma* (Figure 5.1).

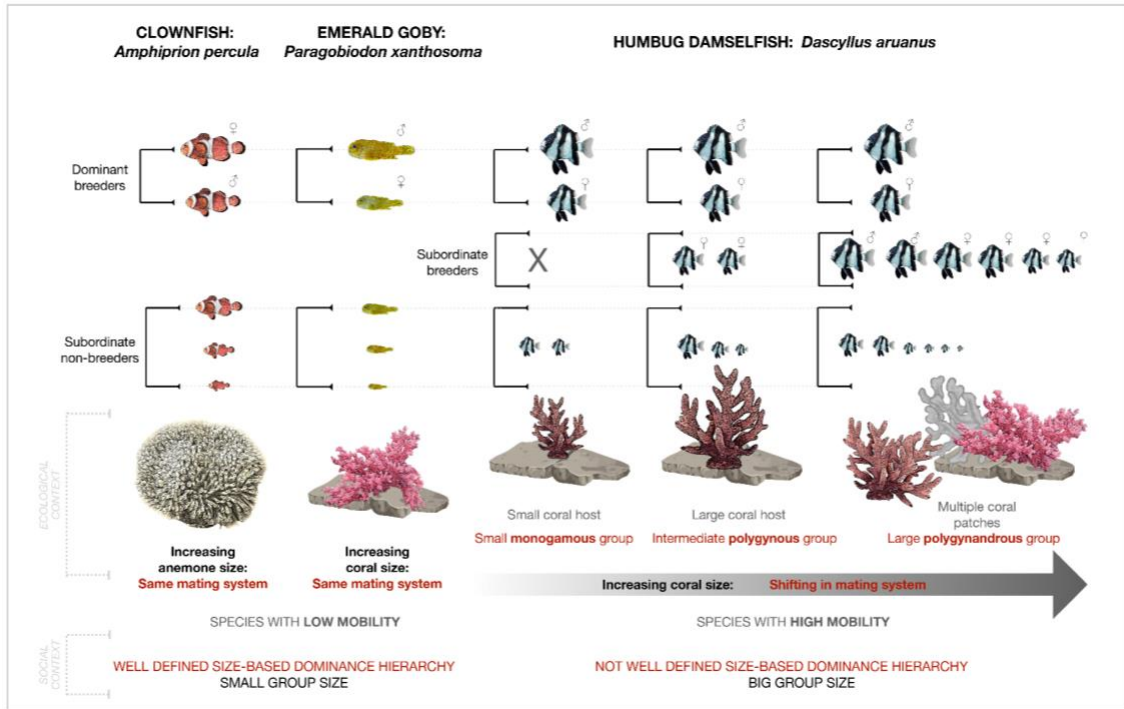
Here we use social groups of the humbug damselfish *Dascyllus aruanus* to test whether cooperation and conflict are influenced by ecological constraints. *D. aruanus* is an ideal model for this because its social system is much more variable and individuals more plastic than other well-studied marine fishes (Figure 5.1), creating the potential for us to test theoretical predictions by manipulating ecological context and examining a variety of responses. *D. aruanus* is widespread throughout the Indo-Pacific region, living in social groups in close association with colonies of certain branching corals (Holbrock

et al., 2000; Sale, 1971). Within each discrete coral patch there is a single group of mostly non-relatives (Sale 1971; Buston et al., 2009). Within each group there is a weakly-defined size-based dominance hierarchy (APPENDIX): the largest individual(s) is male (are males); the intermediate individuals are females; and the smallest individuals are non-breeders (Coates, 1980; Cole, 2002; Asoh, 2003; Wong et al., 2012). The number of individuals in the group increases as coral size increases, and the mating system shifts from monogamy to polygyny to polygynandry and the number of non-breeders increases, with increasing group size (Fricke, 1980; Wong et al., 2012). The fish are protogynous sequential hermaphrodites (Sale, 1970; Cole, 2002; Asoh, 2003): if a male dies or disappear, a female from the focal group of nearby may change sex and take his place (Fricke and Holzberg, 1974; Coates, 1982) and a non-breeder may become female; if the dominant female dies or disappears, and no females or juveniles individuals immigrate to their group, a small male can revert sex back to female (Kuwamura et al., 2016). Within these groups aggressive interactions, e.g., chases and displays, are frequent and are indicative of conflict (Table 5.1); submissive and affiliative displays are less frequent and are indicative of peaceful cooperation, because by reducing conflict they will increase the fitness of actor and recipient (Table 5.1; Branconi et al. 2019b; Buston & Balshine 2007; Wong et al. 2007). *D. aruanus* experiences ecological constraints because suitable habitat is limited, it is risky to move between patches of suitable habitat, and residents of occupied habitat actively repel unfamiliar conspecifics (Schmitt and Holbrook, 1999; Jordan et al., 2010; Forrester, 1991). Additionally, differently from other coral reef fish species that have been focal point for studies of social evolution (Figure 5.1; Wong et al.



2010; Mann et al., 2014; Branconi et al. 2020; Rueger et al. 2021a) in *D. aruanus* focal coral size and habitat availability (i.e. number and size of neighboring corals) can influence the social organization within groups, which likely leads to some of the behavioral variability that makes this such an excellent study system.

The main goal of the present study is to test whether cooperation and conflict are influenced by ecological constraints and their manipulation. Therefore, we investigate the effect of manipulating ecological constraints on the frequency of cooperative and conflictive interactions between individuals, and examine how the effect is modulated by natural variation in social and ecological context. In general, we find that levels of cooperation and conflict are dependent on the interaction between ecological constraints, individual traits, social and ecological context. This work tests our current understanding of the role of ecological constraints in causing variation in cooperation and conflict in social groups, and provides new insights into the underlying causes of cooperation and conflict within animal societies.



**Figure 5.1** Schematic of the social organization of the clown anemonefish *Amphiprion percula* (left), the emerald goby *Paragobiodon xanthosoma* (centre), and the humbug damselfish *Dascyllus aruanus* (right). Both *A. percula* and *P. xanthosoma* are characterized by little variation in their social systems: small group size, well-defined size-based dominance hierarchies, high risks of movement (i.e. low/no individual mobility) and low/no variation in their reproductive skew. *D. aruanus*, instead, is characterized by lots of variation in its social system: group size that vary with the size of the coral patch or the degree of coral cover, no well-defined size-based dominance hierarchies, low risks of movement (i.e. high individual mobility) and high variation in reproductive skew.

## 5.3 Methods

### 5.3.1 Study population

For this study, we used a population of *D. aruanus* at Lizard Island Lagoon on the northern Great Barrier Reef, Australia (14°40' S, 145° 27' E). The study was conducted from January to March 2016 at 3 different diving sites: north of Palfrey Island, Bird Islet and Trawler beach. All fieldwork was conducted using SCUBA at depths less than 20 m. Within the dive sites, the reef consisted of areas of rubble and living corals interspersed with extensive areas of sand. In total, we located and mapped 82 corals using underwater GPS from which we selected 18 groups with a minimum number of 7 individuals (n = 204 individuals in n = 18 groups). Each coral was occupied by a single group of *D. aruanus*.

### 5.3.2 Fish collection and tagging

Fish from each group were anesthetized using a clove oil solution (Munday and Wilson, 1997), captured with a slurp gun or hand net, and placed into zip-lock plastic bags to be transferred to the boat. Once onboard, bags were inflated with air, the bags were put in a cooler, and the fish were transported to laboratory facilities at Lizard Island Research Station. The time from catching to release the fish into the aquaria was less than one hour. Each fish was uniquely tagged using topical surgical glue and colored plastic films that were easily detectable by video/photo-cameras (Branconi et al., 2019a). At the

end of the tagging procedure each group was transported back to its original site and released into their home focal coral.

### *5.3.3 Experimental design and data collection*

#### *5.3.3.1 Characterizing social context*

To control for natural variation in social context on social behavior, we characterized the social context of the 18 focal groups. First, to determine an individual's size, the standard length (SL) of each fish, the straight-line distance from the tip of the snout to the base of the caudal fin (Caillet et al., 1986), was measured to 0.1 mm using calipers. Next, to determine relative competitive ability, the size ratio of an interacting pair was estimated as the SL of the actor divided by the SL of the receiver (Buston & Cant, 2006; Wong et al., 2007) (see APPENDIX; Figure A.1). Finally, group size was determined by counting the number of resident fish on the focal host corals and ranged from 7 to 25 fish. Counts were carried out multiple times, in different days, to ensure an accurate estimate of group size in the focal coral and minimize errors related to i) movements of individuals between different coral hosts within their territory or ii) considering transient fish as actual residents. Only groups that showed the same number of individuals across different days were used in our sample.

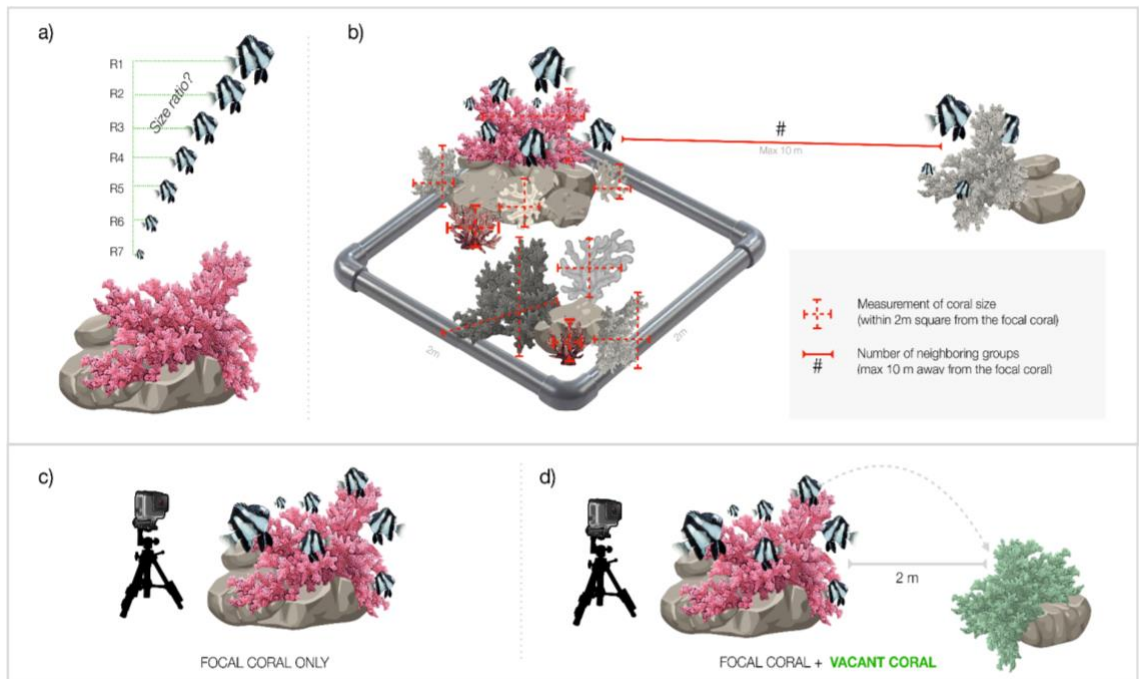
#### *5.3.3.2 Characterizing ecological context*

To control for natural variation in ecological context on social behavior, we characterized the ecological context of the 18 focal groups. First, to estimate territory size

of each group, the size of each focal coral was determined by measuring the length, width, and height of the coral colony using a measuring tape and calculating the ecological volume (Shaish et al., 2007). Then, to estimate habitat availability surrounding groups, the size of neighboring branching corals within 2m square from the focal coral was estimated in the same way and the size of all neighboring corals was summed (Fig.5.2b) (Sale, 1971). Finally, to estimate local density, the number of neighboring groups of *D. aruanus* was determined by counting the groups present on other corals up to 10 m away (Fig. 5.2b; the distance was measured from the focal coral, creating a circle with 10 m radius, to other groups). The areas surveyed for corals and fish groups were different for logistical reasons — we did what was feasible on SCUBA in the time available.

#### 5.3.3.3 *Manipulating ecological constraints*

After characterizing the social and ecological context of our focal groups, we experimentally relaxed individuals' ecological constraints by adding a vacant coral placed two meters away from the focal coral (see Wong 2010 and Branconi et al. 2020 for example). For the selection of the vacant coral, we surveyed the coral population and identified a small number of corals that were healthy and movable (i.e., attached to small rocks). One of these corals (*Seriatopora caliendrum*; ecological volume: 16880.64 cm<sup>3</sup>), was collected and used as vacant coral — corals of this size and species were regularly used by *D. aruanus*.



**Figure 5.2** Schematic of the experimental design and data collection: **a)** characterization of the social context, i.e. individual standard length, size ratio, and group size; **b)** characterization of the ecological context, i.e. ecological volume of each focal coral (in pink), ecological volume of the neighboring branching corals (in grey) within 2m square, and number of neighboring groups present on other corals within 10 m radius; **c-d)** manipulation of the ecological constraints by the addition of a vacant coral (in green) placed 2 meters away from the focal coral (in pink); the collection of the behavioral data was performed through the analysis of video recording and the order of the videos of the groups recorded with and without the vacant coral was randomized.

#### *5.3.3.4 Measuring cooperation and conflict*

Using GoPro Hero 3 and Hero + ([www.gopro.com](http://www.gopro.com)) cameras fixed on tripods, we conducted paired video recordings of each group (Figure 5.2b); each group was recorded twice, each time for a duration of 30 minutes, between 10:00 and 16:00 hours. The camera was placed at 1 m from the colonies. The order of the paired recordings was randomized and recordings were made in quick succession, one after the other. The video recordings commenced one hour after the release of the group into the focal coral to allow acclimation of the fish. Behavioral data obtained from the video recordings of the same group taken the same day with and without the vacant coral present were compared by the same observer (R.B). Videos were watched starting 4 minutes after the beginning of the recording to account for the time necessary for the scuba diver to leave the area. Social behaviors (conflictive and peacefully cooperative interactions Table 5.1), were scored along with the identity of the individuals performing (i.e., actor) and receiving (i.e., receiver) the behaviors. All the videos were examined using the program VLC Media player 2.1.3 ([www.vlc-media-player.en.uptodown.com](http://www.vlc-media-player.en.uptodown.com)). The behavioral data relating to all groups was used to calculate the time difference between interactions within each category of social behaviors for each individual fish. We measured a total of 27851 conflictive interactions (mean number of interactions per individual  $\pm$  SD =  $137 \pm 131.37$ ) and 1838 cooperative interactions (mean number of interactions per individual  $\pm$  SD =  $9 \pm 11.95$ )

**Table 5.1** Summary of the social behaviors —conflictive and cooperative interactions — used in this study. Conflictive interactions are considered those that involve potential or actual harm to another individual within the group. Peacefully cooperative interactions are considered those that promote conflict resolution and reduce costs of conflict between group members.

| BEHAVIORAL CATEGORY  | DESCRIPTION   |
|--|---|
| <p><b>CONFLICTIVE INTERACTIONS</b></p> <p><i>between group members</i></p>           | <p>AGGRESSIVE BEHAVIORS:</p> <ul style="list-style-type: none"> <li>• Chasing (<i>focal fish quickly swims/darts towards another fish</i>)</li> <li>• Biting (<i>focal fish bites another fish</i>)</li> <li>• Aggressive display (<i>focal fish raise all fins and swim upwards to another fish and then takes an oblique position and quivers and finally swims in parallel</i>)</li> <li>• Ram (<i>focal fish makes contact with another fish using the head or mouth region, but no obvious bite is taken -jaws remain closed-</i>)</li> </ul>  |
| <p><b>PEACEFULLY COOPERTIVE INTERACTIONS</b></p> <p><i>between group members</i></p> | <p>SUBMISSIVE BEHAVIORS:</p> <ul style="list-style-type: none"> <li>• Flee (<i>focal fish quickly swims away from another fish as a response to received aggressive behaviors</i>)</li> <li>• Hiding (<i>focal fish quickly swims inside coral branches or holes into the rocks to place itself out of another fish's view as a response to received aggressive behavior</i>)</li> <li>• Submissive display (<i>the head of the focal fish is directed downwards, sometimes entirely vertical, the tail is upwards and the whole body trembles</i>)</li> <li>• Head down (<i>the head of the focal fish is directed downwards, sometimes entirely vertical, the tail is upwards</i>)</li> </ul> <p>AFFILIATIVE BEHAVIORS:</p> <ul style="list-style-type: none"> <li>• Following a group member (<i>focal fish follows another one within the group</i>)</li> <li>• Meeting a group member (<i>focal fish meets another one and swim together</i>)</li> </ul> |



5.3.3.5 *Effect of ecological constraints on the time difference between conflictive and cooperative interactions*

Behavioral data was analyzed by using mixed model analysis with the *lme4* package (Bates, Maechler and Bolker, 2012) in R (R Core Team, 2020). To determine whether the frequency of social behaviors (conflictive and cooperative interactions) varies with respect to ecological constraints when controlling for ecological and social context, we used two generalized linear mixed effect models (GLMM) with gaussian error term (*log link*) where the dependent variables were the time difference between interactions within the same category of social behavior: i) conflictive, ii) peaceful cooperative. The response variables “time difference in seconds between interactions” were log transformed to account for a non-normal distribution. The primary independent variable was treatment (“focal coral only” v. “focal coral plus vacant coral”, categorical). To investigate potential effects of social context on social behaviors social predictors, i) actor’s size (numeric, scaled), ii) actor/receiver size ratio (numeric, scaled) and iii) group size (numeric, scaled) were included in the models as fixed effects and as two-way interactions with treatment. To investigate potential effects of ecological context on social behaviors ecological predictors, i) the ecological volume of the focal coral (numeric, scaled), ii) the total ecological volume of neighboring corals (numeric, scaled) and iii) the number of neighboring groups (numeric, scaled) were included in the models as fixed effects and as two-way interactions with treatment. To control for the number of individuals with which any fish can interact, group size (minus one, since there are no self-interactions in this context) was also included as an offset in the models i.e. *log*

(*group size - 1*). Group ID, actor ID and receiver ID (crossed random intercepts) were also included in the models to control for the lack of independence between multiple measures from the same fish and same group. Significance tests for GLMMs were performed by likelihood ratio tests of the full model with the effect in question against the model without the effect. No homoscedasticity or obvious deviations from normality 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 by testing for outliers and collinearity between variables with *performance* (Luedecke et al., 2020). Conditional and marginal  $R^2$  were calculated using Nakagawa's  $R^2$  in *performance* (Nakagawa & Schielzeth, 2013; Johnson, 2014; Nakagawa et al., 2017). For some models the random term variance component was zero and conditional  $R^2$  could not be calculated. This had no effect on the model components reported here (Pasch et al., 2013). In all cases the full model with all hypothesized main effects and interaction effects, is reported. Importantly, a decrease of the response variable “time difference in seconds between interactions” indicates an increase of the overall frequency of cooperative and conflictive interactions, while an increase of it, indicates a decrease of the overall frequency of these interactions.

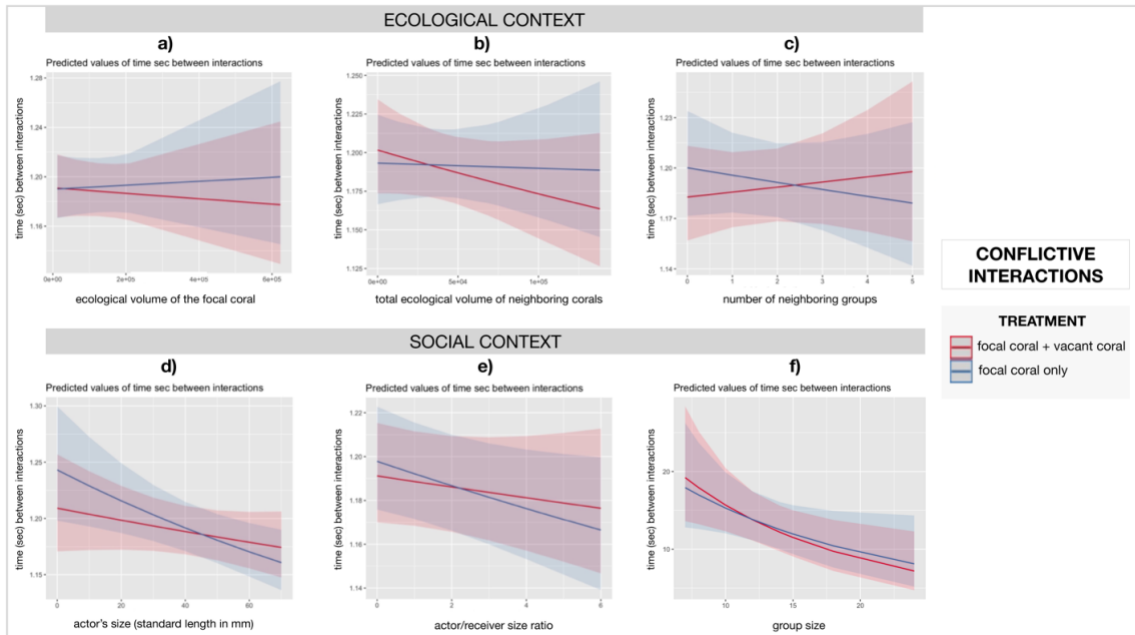
## 5.4 Results

### *5.4.1 Effects of ecological constraints on conflictive interactions*

Our results show that the effect of experimental manipulation of ecological constraints (by relaxing habitat availability with the addition of a vacant coral) on conflictive interactions depends on the current ecological and social context surrounding groups and individuals (Figure 5.3, panels a-f). Regarding the ecological context (Figure 5.3, panels a-c), the frequency of conflictive interactions was dependent on the statistical interaction between the experimental treatment and focal coral ecological volume (log estimate ( $\pm$  standard error (s. e.)) =  $0.029 \pm 0.008$ ,  $\chi_{(1)}=14.207$ ,  $p < 0.001$ ), ecological volume of the neighboring corals (log estimate ( $\pm$  s. e.) =  $0.049 \pm 0.008$ ,  $\chi_{(1)} = 36.454$ ,  $p < 0.001$ ), and number of neighboring groups (log estimate ( $\pm$  s. e.) =  $-0.055 \pm 0.009$ ,  $\chi_{(1)} = 40.675$ ,  $p < 0.001$ ). Specifically, both the slope of the relationship between size of the focal coral and time difference between conflictive interactions (Figure 5.3a) and the slope of the relationship between size of the neighboring corals and time difference between conflictive interactions (Figure 5.3b) were more negative when the additional vacant coral was present. Therefore, when ecological constraints were relaxed (vacant coral present), conflictive interactions became more frequent with increasing sizes of the focal coral and the neighborhood corals (Figure 5.3 a & b).

In contrast, the slope of the relationship between number of neighboring groups and time difference between conflictive interactions (Figure 5.3c) was more positive

when the additional vacant coral was present indicating that, when ecological constraints were relaxed, conflictive interactions became less frequent with an increase in the number of neighboring groups. Regarding the social context (Figure 5.3, panels d-f), the frequency of conflictive interactions was dependent on the interaction between the experimental treatment and actors' size (log estimate ( $\pm$  s. e.) =  $-0.029 \pm 0.009$ ,  $\chi_{(1)} = 11.194$ ,  $p < 0.001$ ) and group size (log estimate ( $\pm$  s. e.) =  $0.032 \pm 0.008$ ,  $\chi_{(1)} = 0.772$ ,  $p < 0.001$ ), but not actor/receiver size ratio (log estimate ( $\pm$  s. e.) =  $-0.007 \pm 0.008$ ,  $\chi_{(1)} = 15.555$ ,  $p = 0.379$ ). More specifically, the slope of the relationship between actors' size and time difference between conflictive interactions (Figure 5.3c) was less negative when the additional vacant coral was present indicating that, when ecological constraints were relaxed, conflictive interactions increased in frequency more slowly with increasing actors' size (Figure 5.3c). The interaction between group size and the presence/absence of the vacant coral is harder to interpret. Taken together the fixed effects explained 20% of the variance ( $R^2_c = 0.246$ ,  $R^2_m = 0.201$ ).

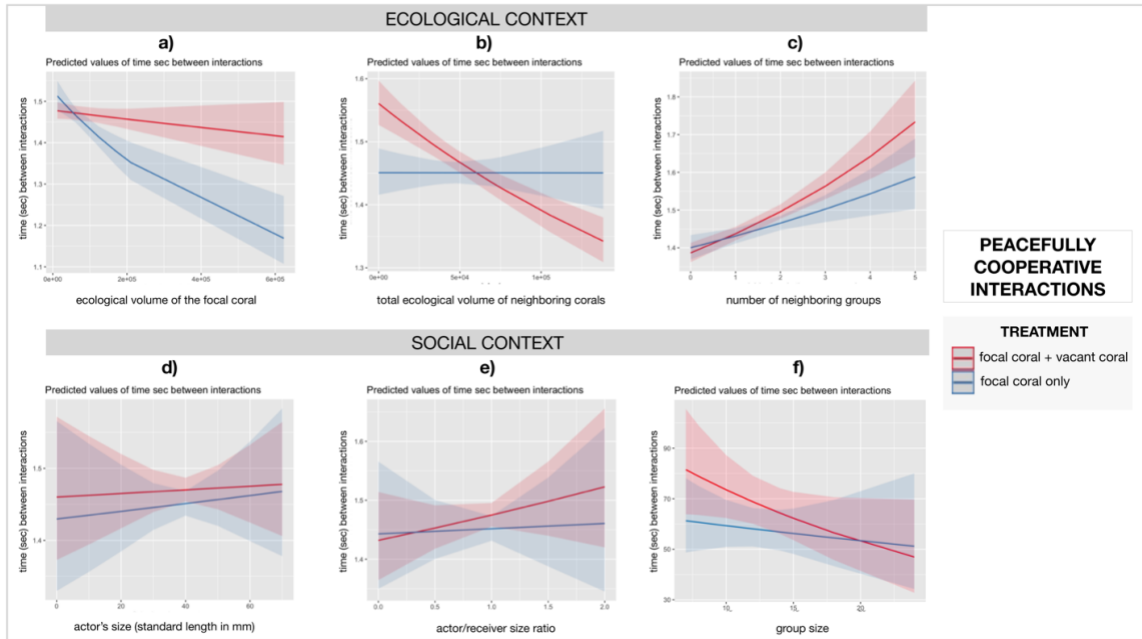


**Figure 5.3** Comparison of the effects of ecological (panels a-c) and social (panels d-f) context on time difference between conflictive interactions in *Dascyllus aruanus* under natural and experimental conditions; **a)** effect of ecological volume of the focal coral; **b)** effect of total ecological volume of the neighboring corals; **c)** effect of number of neighboring groups; **d)** effect of actor's size; **e)** effect of actor/receiver size ratio **f)** effect of group size. Blue line represents natural conditions. Red line represents experimental conditions when ecological constraints are relaxed. Lines represent estimated best fits from models and shading represents 95% confidence intervals.

#### 5.4.2 *Effects of ecological constraints on cooperative interactions*

Our results show that the effect of the experimental manipulation of ecological constraints (by relaxing habitat availability with the addition of a vacant coral) on peacefully cooperative interactions depends only on the current ecological context surrounding groups and individuals, and not the social context (Figure 5.4, panels a-e). Regarding the ecological context (Figure 5.4, panels a-c), the frequency of peacefully cooperative interactions was dependent on the statistical interaction between the experimental treatment and focal coral ecological volume (log estimate ( $\pm$  standard error (s. e.)) =  $-0.124 \pm 0.041$ ,  $\chi_1 8.958$ ,  $p=0.003$ ) and ecological volume of the neighboring corals (log estimate ( $\pm$  s. e.) =  $0.115 \pm 0.039$ ,  $\chi_1 = 8.289$ ,  $p= 0.004$ ) but not the number of neighboring groups (log estimate ( $\pm$  s. e.) =  $-0.089 \pm 0.049$ ,  $\chi_1 = 3.411$ ,  $p = 0.065$ ). More specifically, the slope of the relationship between ecological volume of the focal coral and time difference between peacefully cooperative (Figure 5.4a) was more positive when the additional vacant coral was present indicating that, when ecological constraints were relaxed, the frequency of peacefully cooperative interactions increased less with increasing sizes of the focal coral (Figure 5.4a). In contrast, the slope of the relationship between ecological volume of the neighboring corals and time difference between peacefully cooperative interactions (Figure 5.4b) was more negative when the additional vacant coral was present indicating that, when ecological constraints were relaxed, the frequency of peacefully cooperative interactions increased more with increasing size of the neighborhood corals (Figure 4b). Regarding the social context (Figure 5.4, panels d-

f), the frequency of peacefully cooperative interactions was not dependent on the interaction between the experimental treatment and actors' size (log estimate ( $\pm$  s. e.) =  $0.001 \pm 0.058$ ,  $\chi_1 = 0.004$ ,  $p = 0.982$ ), actor/receiver size ratio (log estimate ( $\pm$  s. e.) =  $-0.047 \pm 0.048$ ,  $\chi_1 = 0.979$ ,  $p = 0.323$ ) or group size (log estimate ( $\pm$  s. e.) =  $0.029 \pm 0.046$ ,  $\chi_1 = 0.408$ ,  $p = 0.523$ ). (Figure 5.4e). For the whole model the random term variance component was zero and conditional  $R^2$  could not be calculated. The fixed explained 5% of the variance ( $R^2_m = 0.053$ ).



**Figure 5.4** Comparison of the effects of ecological (panels a-c) and social (panels d-f) context on time difference between peacefully cooperative interactions in *Dascyllus aruanus* under natural and experimental conditions; **a)** effect of ecological volume of the focal coral; **b)** effect of total ecological volume of the neighboring corals; **c)** effect of number of neighboring groups; **d)** effect of actor's size; **e)** effect of actor/receiver size ratio **f)** effect of group size ions. Blue line represents natural conditions. Red line represents experimental conditions when ecological constraints are relaxed. Lines represent estimated best fits from models and shading represents 95% confidence intervals.



## 5.5 Discussion

To test the hypothesis that cooperation and conflict will be influenced by ecological constraints, we investigated the effect of a manipulation of ecological constraints on conflict and peaceful cooperation in the humbug damselfish *Dascyllus aruanus*. We show that the effect of the manipulation of ecological constraints on conflictive interactions depends on both ecological and social context, while the effect of the manipulation on peacefully cooperative interactions depends only on the ecological context.

### 5.5.1 Conflict

In general, the ecological constraints hypothesis predicts that conflict will increase in groups as ecological constraints are relaxed, and the magnitude of the effect will be modulated by individual traits, social context, and ecological context (Emlen; 1982). The basic rationale for this is that, as individuals outside options improve, they will be more demanding of resources within the group and conflict will increase, up until the point that all individuals' demands cannot be met and the group dissolves via forcible evictions or voluntary departures (Buston & Zink 2009; Johnstone & Cant 2009). Our results suggest that in large focal corals (but not small focal corals), and in the presence of larger/more neighboring corals (but not smaller/fewer neighboring corals), and in bigger groups (but not smaller groups) there was more conflict between group members when ecological constraints were experimentally relaxed. The effect of the number of neighboring groups was more ambiguous: in the presence of few neighboring groups

there is more conflict between group members when ecological constraints were experimentally relaxed, but in the presence of many neighboring groups there is more conflict under control conditions. Finally, small fish (but not big ones) initiated more conflictive interactions when ecological constraints were experimentally relaxed. Taken on balance, these results suggest that some individuals within some groups engage in more conflict when ecological constraints are relaxed and that the presence of a vacant coral can affect the frequency of competitive interactions in certain ecological and social contexts but not others. A possible mechanism to explain these results comes from the anecdotal evidence that during the experimental manipulation, in some groups, some individuals (usually the bigger ones) were moving between the focal coral and the vacant coral, suggesting that relaxing ecological constraints might induced temporary group dissolution. Importantly, the absence of some individuals from the focal group could be the driver of the increased frequency of conflictive interactions between certain individuals (especially the smallest ones left in the focal corals for example). In addition, anecdotal evidence suggests also that the occurrence of inter-group interactions (that in turns could be influenced by the size of the focal corals, the presence of neighboring corals and the number of neighboring groups) might have an important role in determining within group conflict.

To our knowledge only a few studies have investigated the effect of manipulating ecological constraints on within group conflict. Tibbetts and Reeve (2008), for example, reduced ecological constraints in paper wasps (*Polistes dominula*) by providing them an

independent breeding opportunity in the form of an orphaned adoptable nest comb. That study showed a decrease of aggressive interactions between group members when the ecological constraints were experimentally relaxed, but with a differential response depending on individuals' rank. Heg and Taborsky (2010), instead, manipulated the predation risk in the cooperative breeding cichlid *Neolamprologus pulcher* by releasing no predator, a medium- or a large-sized fish predator inside underwater cages enclosing natural groups. Interestingly, they found no evidence that a manipulation of the predation risk affected the overall level of within-group conflict. Furthermore, Liu et al. (2020), using the Asian burying beetle *Nicrophorus nepalensis* experimentally manipulated the presence or absence of blowfly competition surrounding groups. They demonstrated that individuals in harsh environments characterized by high competition invest less in social conflict than individuals of the same ranks in environments with less competition. Taken together, the results of these studies suggest that the manipulation of ecological constraints can affect conflict within certain social groups but not others, potentially with a different impact depending also on individuals' rank. In general, these studies underline that there is not a universal response of animal social groups to different ecological constraints and that the effects of an experimental manipulation, if any, can vary dramatically across different species and between types of ecological constraints.

### 5.5.2 Cooperation

In general, the ecological constraints hypothesis predicts that cooperation will decrease in groups as ecological constraints are relaxed, and the magnitude of the effect will be modulated by individual traits, social context, and ecological context (Emlen; 1982). The basic rationale is that, as individuals outside options improve, they will be less willing to engage in cooperation within the group. Our results suggest that in large focal corals (but not in small ones), and in the presence of more neighboring groups (but not few neighboring groups) there was less cooperation between group members when ecological constraints were experimentally relaxed. The effect of the size of neighboring corals was more ambiguous: in the presence of small/few neighboring corals (but not larger/more neighboring corals) there was less cooperation when ecological constraints were relaxed, but in the presence of large/more neighboring corals there was less cooperation under control conditions. Taken on balance, these results suggest that individuals in some groups engage in less cooperation when ecological constraints are relaxed. Once again, anecdotal evidence suggested that our experimental manipulation potentially induced a temporary group dissolution in some groups, and that the absence of certain individuals from these groups could be the driver of the decreased frequency of cooperative interactions as well as the occurrence of inter-group interactions.

To our knowledge, only a few studies have investigated the effect of manipulating ecological constraints on within group cooperation. For example, Bergmüller et al. (2005), conducted a manipulation of the ecological constraints by creating vacant breeding sites in the cooperative breeding cichlid *Neolamprologus*

*pulcher*. That study showed a decrease of the amount of cooperative interactions performed by subordinate individuals when the vacant breeding sites were presented. In addition, Heg and Taborsky (2010) conducted another study on the same species and experimentally altered the predatory risk surrounding groups. Interestingly, they demonstrated an increase of the cooperative efforts carried out by large subordinates when the risk of predation was experimentally incremented. Furthermore, Lindstedt et al. (2018) by using the gregarious social herbivore *Diprion pini* pine sawfly, investigated the effect of attack intensity (manipulated by repeatedly harassing larvae to produce a chemical defence) on cooperation efforts carried by individuals. That study demonstrated that the number of larvae participating in group defence decreased under high attack intensity, and this reduction was more pronounced on the low resin compared with the high resin diet. Lastly, Liu et al. (2020) experimentally manipulated the presence or absence of blowfly competition surrounding groups in the Asian burying beetle *Nicrophorus nepalensis*. The results of that study showed that, in harsh environments (i.e. high competition), all individuals invested more in cooperative interactions than individuals of the same ranks in benign environments (i.e. low competition) but low-ranking subordinates increased their investment in cooperation proportionally more than high-ranking dominants, suggesting that subordinates contribute relatively more when facing environmental challenges. Taken together, the results of these studies suggest that the manipulation of ecological constraints can influence the adaptive value of cooperative interactions and maintain variation in the frequency of cooperation between individuals within social groups but that these effects can vary across different species and contexts.

### 5.5.3 Dispersal

While relatively few studies have directly investigated the effect of manipulating ecological constraints on cooperation and conflict, many more studies have investigated the effect on the dispersal decision of subordinates and group break-up (e.g., Pruett-Jones & Lewis, 1990; Walters et al, 1992; Heg et al., 2004; Bergmüller et al., 2005; Halliwell et al., 2017; Brouwer & Cockburn 2020), which can be considered an indicator of increased conflict and a reduction in cooperation. In general dispersal of subordinates is more likely when ecological constraints are relaxed, and the likelihood varies with social and ecological context. For example, Komdeur (1992) demonstrated the dispersal decisions of subordinates depended on territory quality. Others have demonstrated that variation in dispersal decisions depends on variation in the risks of movement even when ecological constraints are relaxed by provision of vacant habitat (Wong, 2010; Branconi et al. 2020). Similarly, in the present study we show that in *D. aruanus* the manipulation of ecological constraints by the addition of a vacant coral induced a temporary group break-up (only anecdotal evidence), increased conflict and decreased cooperation in some individuals, some groups, and some contexts.

### 5.5.4 Conclusion

In sum, our work investigated the effect of the manipulation of ecological constraints on both within group cooperation and conflict in coral reef fish societies by measuring a range of different ecological and social factors. We find that relaxed ecological constraints result in increased conflict and decreased cooperation in some

individuals, some groups, and some contexts. The results of this study, importantly, suggest that to truly understand how ecological constraints interact with variation in individual traits, social context and ecological context to influence cooperation and conflict within groups and group stability, it is imperative to adjust theoretical models to incorporate also variation in individual body size, territory size, habitat availability, conspecifics density and inter-group interactions. To date, the general understanding of how different ecological contexts influence cooperation and conflict between individuals is still largely unexplored and has the potential to reveal important insights about the evolution of non-breeding strategies, individual dispersal decisions and group stability in any animal society, including humans, opening the opportunity to conduct informative comparative analyses across different systems and taxa.

## **5.6 Acknowledgements**

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number: G14/ 37263.1). The research was supported by one summer and one annual Warren McLeod Fellowship awarded by Boston University to R. Branconi and one University of Wollongong Target Grant awarded to M. Wong.



## CHAPTER SIX

### Discussion

#### 6.1 Contributions of this Dissertation to the study of social evolution

A major goal of evolutionary biology is to understand the evolution of social groups in which some individuals forgo their own reproduction and help others to reproduce. The evolutionary framework for understanding these groups has focused on the benefits acquired via kin selection and territory inheritance and the costs of pursuing alternative options due to ecological and social constraints. My dissertation research investigates the effect of ecological and social constraints on social evolution using two marine social systems, the clown anemonefish *Amphiprion percula* and the humbug damselfish *Dascyllus aruanus*. My research tests the robustness of current theories and generates new insights into the evolution of cooperation and non-breeding behaviors in any animal social groups.

In the first part of my dissertation, I explored how ecological and social constraints influence the evolution of non-breeding strategies in *A. percula*, the main focus of the first two decades of social evolution research in marine fishes. Using three manipulation experiments in the wild, I explored why clownfish non-breeders engage in peaceful cooperation, waiting to inherit breeding positions, rather than engaging in one of two alternative non-cooperative options: i) the outside option (i.e. leaving to breed elsewhere); and ii) the inside option (i.e. contesting to breed at home) (Chapter 2). I demonstrated that clownfish non-breeders will disperse, when ecological constraints (risk

of mortality during dispersal) are experimentally weakened, and will contest, when social constraints (risk of eviction during contest) are experimentally relaxed. These results showed that it is the combination of ecological and social constraints that promote the evolution of non-breeding strategies in the clownfish *A. percula* and, importantly, served as groundwork from which I extended the framework for the study of social evolution to another marine fish with a more complex social system, *Dascyllus aruanus* (Chapters 3, 4 and 5).

In the second part of my dissertation I broadened our understanding of the effect of alternative options in social evolution using the humbug damselfish *D. aruanus*, a species with high individual mobility (Mann et al., 2014), weakly defined size-based dominance hierarchies (Branconi et al., 202X) and relatively low reproductive skew (Wong et al., 2012). First, given the ineffectiveness of standard tagging methodologies on *D. aruanus* (e.g., phenotypic variation, beads and PVC tags, fluorescent elastomer injections), I developed a new method to temporarily identify individuals of this species using colored plastic films and topical surgical glue (Chapter 3; Branconi et al., 2019a). Then, I examined the survival time of the tags at various positions on the body of the fish. The results showed that i) fish size was not a significant predictor of tag life and ii) the optimal tag locations were dorsal anterior (with a median attachment time of 53 hours) and dorsal middle (with a median attachment time of 49 hours). More broadly, these films are easily detectable both directly by human observers and indirectly by video/photo-cameras and could be applied successfully also to other fishes and aquatic organisms (e.g., amphibians) in both marine and freshwater ecosystems, ultimately

helping the advances of future behavioral or ecological studies. Next, I developed a method essential for data collection and behavioral observations of *D. aruanus* by i) generating an ethogram describing the behavioral repertoire of the species and ii) investigating how these fish responds to the presence of human observers (effect of scuba diver presence-absence) and how the method of data collection (directly by a scuba diver vs. indirectly via video camera) may affect the quality of behavioral data (Chapter 4; Branconi et al. 2019b). First, for the production of the ethogram, I identified 36 different behaviors that were then classified into five main categories of behavior (aggressive, social, maintenance, reproductive and submissive). Then, I demonstrated that scuba diver presence had only subtle effects on fish behavior and that the efficiency of the method of scoring fish behavior depended on the behavior under consideration (those behaviors that occur in close proximity to the corals were scored more effectively directly by a scuba diver while those that are performed in a more rapid or repetitive fashion were scored more effectively indirectly via video camera). These results provide a foundation for future behavioral research on *D. aruanus* and also other fishes where scuba divers or video cameras are the prevalent means of data collection.

Subsequently, returning to the topic of social evolution, I examined if the intensity of conflict and cooperation within groups varies with respect to the ecological constraints under natural and experimental conditions (Chapter 5). Specifically, I tested the hypothesis that conflict will increase and cooperation will decrease when individuals outside options are improved, i.e., ecological constraints are relaxed. I experimentally increased individuals' outside options by adding a vacant coral (i.e. the outside option)

and measured conflict and cooperation within groups in the presence and absence of the outside option. Further, I examined whether the effect of the manipulation was modulated by the social and/or ecological context. I demonstrated that relaxed ecological constraints interact with variation in social context and ecological context to influence conflict and cooperation within social groups. On balance, the results indicate that in some individuals, in some social contexts, in some ecological contexts, the relaxation of ecological constraints results in an increased frequency of conflictive interactions and reduced frequency of cooperative interactions. These results expand our understanding of the evolution and maintenance of sociality in a species with a complex social system.

Together, the results of my dissertation research show how, in both *A. percula* and *D. aruanus*, ecological and social context act in concert influencing social dynamics and group stability, highlighting the importance of studying the evolution of cooperation and non-breeding behaviors in taxonomically varied social systems. My dissertation research, in fact, underlines the importance of using marine taxa as model systems (in addition to better-known terrestrial species) to investigate how ecological and social constraints influence social evolution. First, the use of marine fishes offers us the possibility to conduct manipulative experiments (e.g. Chapter 2, Chapter 5) that are not necessarily possible in other social vertebrates. Second, while providing important insights into the general understanding of the evolution of cooperation and non-breeding behaviors in animal societies, the use of marine fishes helps us also detecting key differences between aquatic and terrestrial systems. In coral reef fish, differently from terrestrial societies, i)

kin selection does not play a central role to explain the evolution of sociality and ii) alloparental care is absent, but other forms of cooperative actions, such as growth regulation, can be taken by subordinate group members (Buston, 2003a; Buston & Cant, 2006; Wong et al., 2008a, Branconi et al., 2020). With the addition of new studies on marine taxa, therefore, cooperative breeding theory will progressively encompass a continuum of taxonomically varied cooperative social systems, generating a more broad and solid framework for understanding social evolution.

### **6.1.2 Future Directions**

Several lines of future research will advance our understanding of how ecological and social constraints influence social evolution in animal societies. First, we can use social network analysis to investigate not just the frequency of interactions but also the structure of interactions. The generation of social networks and the study of the emerging metrics (i.e. formal descriptors for characterizing social groups), for example, could help us investigating the relationship between individuals' interactions and group structure (Wey et al., 2008; Croft et al., 2008; Krause et al., 2009; Croft et al., 2016; Farine & Withehead, 2015; Krause et al., 2015; Gokcekus et al, 2021), therefore clarifying if different ecological and social context alters not just the frequency of interactions within groups (as I showed in Chapter 5), but also groups' organization.

Another interesting line of future research will be investigating the effects of ecological and social constraints at the individual level. In fact, not all individuals

respond in the same way to different ecological and social context, as suggested by the result of the experiments on *A. percula* in Chapter 1, where some individuals returned home while others did not and some individuals contested while others did not, and *D. aruanus* in Chapter 5, where some individuals engaged in more conflict and less cooperation while others did not. One interesting hypothesis to explain this variability is that this variation could be due to different personality traits of individuals (defined as inter-individual differences in behavior that are consistent over time and across contexts; Réale et al., 2007). Previous studies have already demonstrated the existence of personality traits (e.g., boldness, aggressiveness, shyness, sociability, and parenting behaviors) in different coral reef species (e.g. Wong et al., 2013; Schmiede et al., 2017; Barbasch & Buston, 2018) and future studies will investigate how individual personality traits influence individuals' decisions in fish societies, and how different combinations of individuals' personality traits may influence the structure and function of these societies.

Finally, another important aspect to consider to advance our understanding of how ecological and social constraints influence social evolution in animal societies will be to investigate how mutualistic partners, e.g. the cnidarian hosts for coral reef fishes but also the plant or fungal mutualists for ants for example, may influence individuals' response to different ecological and social contexts. In fact, as suggested by the results of the second experiment in Chapter 1 where two-thirds of clownfish non-breeders returned home when relocated to an alternative anemone at 0.5 m, synergistic effects between the cnidaria host and resident fish may be an important factor influencing individuals'

choices and group stability (e.g. influencing decisions relative to i) the dispersion of the non-breeders to breed elsewhere, ii) the tolerance of non-breeders by the breeding individuals). In addition, since the host's susceptibility to environmental disturbances (nowadays highly impacted by climate change and related stressors) may have severe consequences for the social organization of many marine species by directly influencing the animals (physiologically and behaviorally) or by altering their habitat and mutualistic partners, more studies are needed to test these ideas.

In conclusion, the use of coral reef fish for the study of social evolution will undoubtedly yield new and fascinating insights that could potentially go well beyond the limits of the marine realm and be extended to the study of evolution of cooperation and non-breeding behaviors of any other animal social group.

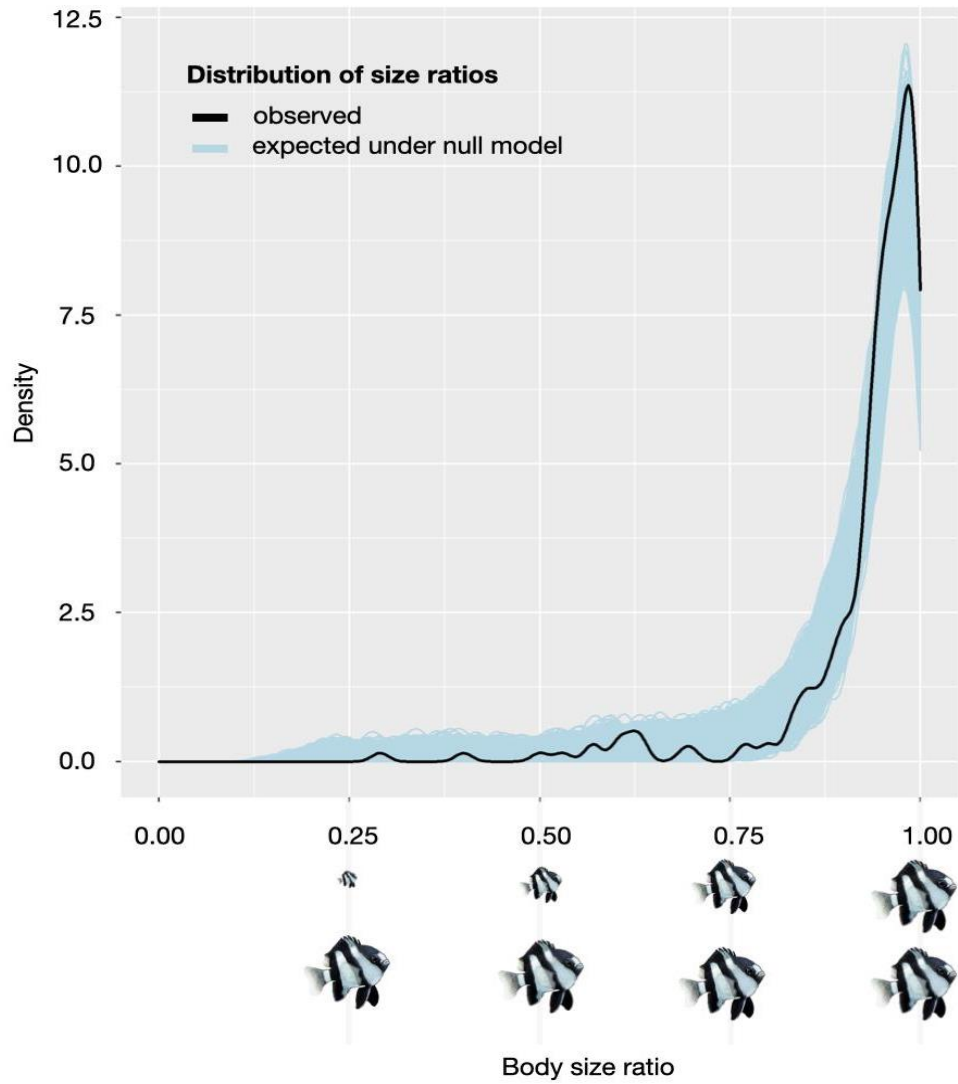
**APPENDIX****Supplement for chapter 5***Investigation of the pattern of the intraspecific size ratios of *D. aruanus**

To investigate whether there is a well-defined, non-random, size ratio between individuals adjacent in rank, as seen in some other coral reef fishes (e.g., Buston & Cant, 2006; Wong et al., 2007) individuals were ranked (1–25) based on their size relative to other individuals within the same group, with the largest being ranked 1. An individual of rank  $N$  was considered to be dominant to all individuals with ranks greater than  $N$ , and subordinate to all individuals with ranks less than  $N$ . We determined the ratio of SL of individuals adjacent in rank within each group: (SL rank  $N+1$ /SL rank  $N$ ). Therefore, we generated the observed distribution of size ratios between individuals adjacent in rank in 25 groups and compared it to the expected distribution of size ratios under a null model (Buston & Cant, 2009; Strong et al., 1979; Gotelli & Graves, 1996). We obtained an observed distribution of 250 ratios from 25 groups. We created a null distribution of 250 ratios using a Monte Carlo procedure, programmed in R (version 4.0.2). To do this we randomly selected individuals from the pool of 275 available individuals (the same 25 groups) and combined them into groups according to the natural distribution of group sizes found in our sample. We then ranked the individuals in these random groups on the basis of relative size, and calculated the ratio of the size of individuals adjacent in rank. The procedure was iterated 10,000 times, generating an expected random distribution of ratios against which our observed distribution could be compared (Buston & Cant, 2006;



Gotelli & Graves, 1996). Each permutation of the sample was compared using the Kolmogorov–Smirnov test.

The comparison between the two distributions revealed that the observed distribution of ratios was not significantly different from the distribution of ratios generated by the null model (only 6.57% of the Kolmogorov–Smirnov test was significant at 5% alpha level, which suggested that the overwhelming majority of samples from the null distribution was not statistically different from the observed samples; Fig. A.1); therefore, in *D. aruanus* the size hierarchy was considered poorly defined and the social constraints were characterized as relaxed, relative to other species (Buston & Cant, 2006; Branconi et al., 2020).



**Figure A.1** Investigation of the pattern of the intraspecific size ratios of *Dascyllus aruanus*: the observed distribution of 250 size ratios of individuals adjacent in rank (black) and the expected random distribution of 250 size ratios under the null model (light blue) iterated 10.000 times.

**LIST OF JOURNAL ABBREVIATIONS**

|                          |   |
|--------------------------|---|
| Acta Ethol               | Acta Ethologica   |
| Am J Primatol            | American Journal of Primatology                           |
| Am Nat                   | The American Naturalist                                   |
| Am Sci                   | American Scientist  |
| Anim Behav               | Animal Behaviour  |
| Aquac Eng                | Aquacultural Engineering                                  |
| Aquat Conserv            | Aquatic Conservation                                      |
| Behav Ecol               | Behavioral Ecology  |
| Behav Ecol Sociobiol     | Behavioral Ecology and Sociobiology                       |
| Behav Process            | Behavioural Processes                                     |
| Biol Lett                | Biology Letters   |
| Biol Rev                 | Biological Reviews  |
| Biol Rev Camb Philos Soc | Biological Reviews of the Cambridge Philosophical Society |
| Bull Mar Sci             | Bulletin of Marine Science                                |
| Can J Fish Aquat Sci     | Canadian Journal of Fisheries and Aquatic Sciences        |
| Commun Biol              | Communications Biology                                    |
| Conserv Ecol             | Conservation Ecology                                      |
| Curr Opin Behav Sci      | Current Opinion in Behavioral Sciences                    |
| Curr Zool                | Current Zoology   |
| Ecol Lett                | Ecology Letters   |
| Environ Biol Fishes      | Environmental Biology of Fishes                           |
| Environ Manage           | Environmental Management                                  |
| Fish Res                 | Fisheries Research  |
| Front Ecol Evol          | Frontiers in Ecology and Evolution                        |
| Front Mar Sci            | Frontiers in Marine Science                               |
| J Anim Ecol              | Journal of Animal Ecology                                 |
| J Comp Physiol           | Journal of Comparative Physiology                         |
| J Ethol                  | Journal of Ethology                                       |
| J Exp Mar Biol Ecol      | Journal of Experimental Marine Biology and Ecology        |
| J Fish Biol              | Journal of Fish Biology                                   |
| J Fish Wildl Manag       | Journal of Fish and Wildlife Management                   |
| J Helminthol             | Journal of Helminthology                                  |

|                                     |  |
|-------------------------------------|--|
| J North Am Benthol Soc              | Journal of the North American Benthological Society                                |
| J R Soc Interface                   | Journal of the Royal Society, Interface  |
| J Stat Soft                         | Journal of Statistical Software  |
| J Theor Biol                        | Journal of Theoretical Biology   |
| Jpn J Ichthyol                      | Japanese Journal of Ichthyology  |
| Mar Biol                            | Marine Biology   |
| Mar Ecol                            | Marine Ecology   |
| Mar Ecol Prog Ser                   | Marine Ecology Progress Series   |
| Mar Fresh Behav Physiol             | Marine and Freshwater Behaviour and Physiology                                     |
| Mediterr Mar Sci                    | Mediterranean Marine Science   |
| Methods Ecol Evol                   | Methods in Ecology and Evolution   |
| Mol Ecol                            | Molecular Ecology  |
| Nat Commun                          | Nature Communications  |
| Nat Ecol Evol                       | Nature Ecology & Evolution   |
| PNAS                                | Proceedings of the National Academy of Sciences of<br>the United States of America |
| Polar Biol                          | Polar Biology  |
| Proc Natl Acad Sci                  | Proceedings of the National Academy of Sciences of<br>the United States of America |
| Proc R Soc B                        | Proceedings of the Royal Society of London. Series<br>B, Biological Sciences       |
| Proc Royal Soc B                    | Proceedings of the Royal Society of London. Series<br>B, Biological Sciences       |
| Proc Royal Soc London<br>B Biol Sci | Proceedings of the Royal Society of London. Series<br>B, Biological Sciences       |
| Q Rev Biol                          | Quarterly Review of Biology  |
| Quart Rev Biol                      | Quarterly Review of Biology  |
| Raffles Bull Zool                   | Raffles Bulletin of Zoology  |
| Trans Am Fish Soc                   | Transactions of the American Fisheries Society                                     |
| Trends Ecol Evol                    | Trends in Ecology & Evolution  |
| Urban Ecosyst                       | Urban Ecosystems   |
| World Econ                          | The World Economy  |
| Z Tierpsychol                       | Zeitschrift für Tierpsychologie  |

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**CURRICULUM VITAE**

