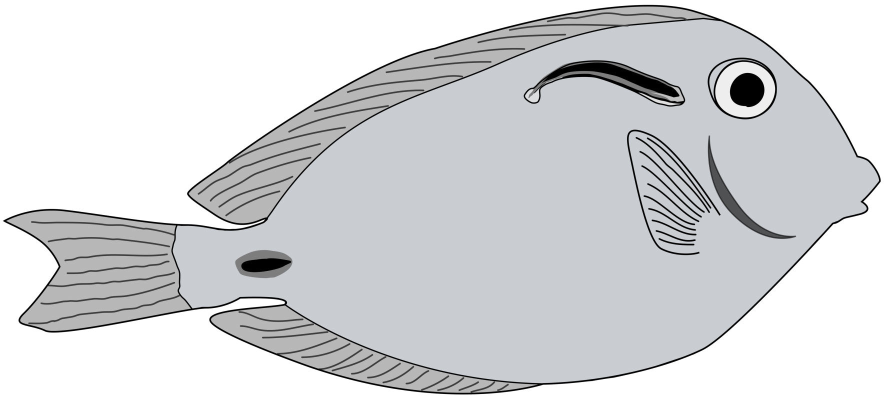
The Dynamics of Cleaner-Client Interactions



Katherine Dunkley (BSc, MSc)
NERC GW4+ funded

Thesis submitted for degree of
Doctor of Philosophy
from Cardiff University
April 2019

Declaration

This work has not been submitted in substance for any other degree or award at this or any other university or place of learning, nor is being submitted concurrently in candidature for any degree or other award.

Signed………………………………………… (candidate) Date……………………

STATEMENT 1

This thesis is being submitted in partial fulfilment of the requirements for the degree of PhD

Signed………………………………………… (candidate) Date……………………

STATEMENT 2

This thesis is the result of my own independent work/investigation, except where otherwise stated. Other sources are acknowledged by explicit references. The views expressed are my own.

Signed………………………………………… (candidate) Date……………………

STATEMENT 3

I hereby give consent for my thesis, if accepted, to be available for photocopying and for inter-library loan, and for the title and summary to be made available to outside organisations.

Signed………………………………………… (candidate) Date……………………

STATEMENT 4: PREVIOUSLY APPROVED BAR ON ACCESS

I hereby give consent for my thesis, if accepted, to be available for photocopying and for inter-library loans after expiry of a bar on access previously approved by the Academic Standards & Quality Committee**.**

Signed………………………………………… (candidate) Date…………………

Acknowledgments

In February 2018 my grandad passed away. He always encouraged and supported my dreams, ambitions and academic achievements, and his passing has left a big hole. I have written this thesis with him always in the back of my mind – he would have loved to have seen it finished.

First, I would like to thank my two supervisors Sarah Perkins and Jo Cable for their help, support and encouragement over the past 4 years. They have brought out the best in me and my work and have helped me with my writing, scientific questioning, fieldwork and career development, and encouraged me to thrive as a scientist, all of which I am grateful for. Jo and Sarah saw the annual Cardiff University undergraduate Tobago field course as a prime opportunity to start collecting a long-term data set on cleaner-client interactions, and I am very thankful that they chose and entrusted me to organise, manage and work with this unique data.

I would also like to thank Dr Christos Ioannou from the University of Bristol, who provided support and advice for the cleaner fish personality work. This was a difficult chapter to write and so I am grateful for his help especially with regards to the statistical analyses. I would also like to thank Associate Professor Ashley Ward for his positive feedback regarding work carried out at the University of Sydney's research centre on One Tree Island, the Great Barrier Reef. He facilitated mine, Jo and Sarah's visit to this magical reef site, and I will always hold fond memories of this Australian fieldwork. Thanks also to Professor Cock van Oosterhout who provided statistical advice for the long-term cleaning patterns chapter.

None of this work would have been possible without NERC GW4+ Doctoral Training funding, and I wish to thank NERC for providing me with this prestigious opportunity. Thanks also to a Cardiff Incoming Visiting Fellows Scheme & International Collaboration Seedcorn Fund awarded to Sarah which funded the Australia fieldwork. Thanks also to following people for assisting me in organising my PhD fieldwork, and for providing in-field support: Neil Cook, Ryan Mohammed, Cock van Oosterhout, Pat Turpin (President of Environment Tobago), Environment Research Institute Charlotteville (ERIC); One Tree Island managers Ainsley Carlin and Paul Baker; Cardiff University Finance members Samantha Phillips and Gareth Hurley.

Across the past 4 years I have had the pleasure of teaching four fish ID lectures to Cardiff University undergraduates prior to the Tobago field course each year. I have loved every minute of this experience and wish to thank all years for their engagement and enthusiasm. These students have also enhanced my fieldwork experience and I also wish to thank them and the previous years' students for collecting the long-term fish abundance data. Thanks also to all the staff that have attended this trip and helped collect the long-term data (see "Data Collection Acknowledgements" section).

Whilst in Cardiff I have been supported by fellow staff and students within the CRIPES and LERKINS research groups and I wish to thank the following for their friendship, coffee break laughter and scientific support: Rhidian Thomas, Alex Stewart, Jo James, Sophie Watson, Josie Jackson, Nia Thomas, Becky Pawluk and Rachel Paterson. I would especially like to thank Kath Whittey and Amy Ellison. Kath was a brilliant field assistant, and without her, this thesis would have been impossible – Kath has always been there for me both in the field, and back home. Thanks also to Amy, who has been my 'agony aunt' over numerous coffees across the years. Last year, I also moved in with Amy and her husband Mark, and words cannot express how grateful I am for this, and how much it has helped me. I am not sure how I would have gotten through the last year of my PhD without them and their dogs – thank you for everything you have done for me.

I would also like to thank my friends who are external to work. Thanks to Tor Jones for welcoming me to Cardiff, letting me live with her, and for providing endless cups of tea. Thanks also to Aimee Robinson, who has always been there for me even when she was going through the most unimaginable of times herself, she is, and will forever be, my greatest friend.

Last but by no means least I would like to thank my family. My partner Alex Boys has made me laugh when times have been hard and encouraged me to take breaks, and my little brother Sam has always acted like a big brother. My mum and dad deserve special thanks for their unconditional love and support. They have helped me both personally and academically and have always put my needs before theirs. This journey has been incredibly mentally challenging and without the support of my family and friends I would not be in this place today:

"Determination today, leads to success tomorrow".

Data Collection Acknowledgements

All data were collected by Katie Dunkley, with the additional help of:

CHAPTER 3

Sarah Perkins and Jo Cable

CHAPTER 4

Kathryn Whittey assisted with *in situ* collection. KD analysed all videos.

CHAPTER 5

Gabrielle Archard, Amy Ellison, Jo Cable, Linda Erlandsson, Fran Hockley, Sarah Perkins, Bettina Schelkle, Jessica Stephenson, Rhidian Thomas, Kathryn Whittey and Raquel Xavier. Cardiff University 2nd year undergraduate students x 8 years.

CHAPTER 6

Gabrielle Archard, Amy Ellison, Jo Cable, Linda Erlandsson, Fran Hockley, Sarah Perkins, Bettina Schelkle, Jessica Stephenson, Rhidian Thomas, Kathryn Whittey and Raquel Xavier. Cardiff University 2nd year undergraduate students x 8 years.

CHAPTER 7

Kathryn Whittey

CHAPTER 8

Kathryn Whittey assisted with camera deployment. KD analysed all videos.

Contents

List of Figures

List of Tables

Thesis Abstract

Mutualisms involve beneficial interactions between species and link every eukaryote on the planet. Despite their pervasiveness, however, it is not clear why these interactions evolved and are so ubiquitously maintained. Cleaning is an iconic mutualism and involves a cleaner species, often a fish, removing ectoparasites and other material from the body of a heterospecific (termed a client): cleaners gain a food source, whilst the clients' health can benefit from parasite removal. This study aimed to contribute to knowledge on the evolution and persistence of mutualisms by asking: how pervasive is cleaning across the animal kingdom, and what factors can influence the pervasiveness (**Chapters 2 – 4**) and dynamics (**Chapters 5 – 8**) of the interaction at an individual, population and species level.

Mutualisms are complex and dynamic interactions, and this study identified a suite of contextual factors that can influence the pervasiveness and patterns of the interaction (**Chapter 2**). *In situ,* the availability of specific food resources, spatial location and the abiotic environment influenced the occurrence of cleaning (**Chapters 3** and **4**). Using a long-term 8 year data set collected on cleaner-client interactions on the same Caribbean reef, **Chapters 5** and **6** showed that even within the same environment, cleaning patterns can vary temporally, spatially and, across and within client identities. **Chapter 6** subsequently found that the abundance and diversity of clients (cleaner choice options), consistently regulated cleaning frequencies. The importance of both cleaner and client choice in governing who interacts with whom and how, was also found, at an individual level (**Chapters 7** and **8**).

Together, this study highlights how sensitive cleaning interactions are to changing contexts, and how their occurrence and functioning, relies on the wide abundance, diversity and variable behaviour of clients. As **Chapter 4** shows, iconic cleaning interactions are not immune to breakdowns, and coral reefs are currently one of the most threatened ecosystems on Earth, experiencing sharp declines in species abundance and diversity. It is not clear whether cleaning will be maintained as a food acquisition behaviour under future environmental conditions.

Chapter 1

An Introduction to Cleaner-Client Interactions

MUTUALISMS

"In nature, nothing exists alone" (Carson 1962) and mutualisms, which involve short- or long-term beneficial interactions between species, are responsible for the diversity and productivity of eukaryotic life on Earth (Leigh 2010; Bronstein 2015). If eukaryotes did not interact with mitochondria, they would struggle to derive energy from food, and without eukaryotic-cyanobacteria mutualisms, primary producers would not exist (Margulis 1993). Land-based primary producers interacting with symbiotic fungi have better access to soil phosphorous, enhancing their growth and survival (Smith et al. 2011), similarly to corals hosting, oxygen producing and waste removing, zooxanthellae algae (Muscatine and Porter 1977). Unquestionably, plants and corals are the key building blocks of diverse and abundant ecological communities (Connell 1978). Within these environments, mutualisms also facilitate a whole host of fitness enhancing traits for species, including reproduction and dispersal (e.g. endofungal bacteria promoting blight fungus, *Rhizopus microsporus,* reproduction, Partida-Martinez et al. 2007, and insects pollinating flowers and dispersing seeds, Bronstein et al. 2006), health enhancing parasite removal (e.g. cleaners eating parasites from clients, Clague et al. 2011b), reduced predation risk (e.g. bobtail squid, *Euprymna scolopes*, and bioluminescent bacteria, *Vibrio fischeri,* counterillumination, Jones and Nishiguchi 2004), and increased foraging gains (e.g. endosymbiotic bacteria and fungi aiding insect digestion, Gibson and Hunter 2010). Mutualistic interactions are thus crucial for key ecological processes, like the nutrient and carbon cycles (e.g. nitrogen-fixing legume-rhizobium interactions, Kiers et al. 2003, and decomposition by fungi-termite interactions, Aanen et al. 2002). Ultimately mutualisms play an unequivocally pivotal role in the structuring and functioning of ecological communities (Stachowicz 2001; Thébault and Fontaine 2010). Despite their extensive occurrence and importance however, explaining the persistence of these beneficial interactions between taxonomically distant species, still remains one of the greatest evolutionary questions (Herre et al. 1999; Kiers et al. 2003; Bronstein 2015).

Nearly 20 years ago, Hoeksema and Bruna (2000) identified three broad unanswered questions on mutualisms: how did mutualisms evolve, how are they maintained and remain mutualistic, and finally how are they affected by the complex communities within which they occur? Despite a large number of theoretical and empirical studies since, these questions are still largely unresolved today: mutualisms are inherently complex and dynamic interactions since they are heavily influenced by the abiotic and biotic context (Chamberlain et al. 2014; Hoeksema and Bruna 2015). Differing contexts control the benefits each partner gains, influencing how they behave (Frederickson 2017). The diversity and dynamics of mutualisms thus makes them a challenging interaction to fully understand. This thesis therefore aims to contribute to all three of Hoeksema and Bruna's (2000) questions, by investigating which factors influence the dynamics and occurrence of an iconic mutualism, the cleaner-client interaction.

CLEANER-CLIENT INTERACTIONS

Most mutualisms are service-resource interactions, where a resource is exchanged for a beneficial act. Service providers can gain a food resource (e.g. ectoparasites or nectar) with the provided service increasing the partners fitness (e.g. disease and parasite control, Arnal et al. 2001, or pollination, Landry 2012). Aside from plant-pollinator interactions, cleaner-client mutualisms are the most referenced service-resource mutualisms, with their charismatic nature often being shown in natural history media. They are also used as one of the model systems for theoretical studies using game theory (Bshary and Bronstein 2011). Iconic cleaner species include the Indo-pacific bluestreak cleaner wrasse (*Labroides dimidiatus*) and oxpecker cleaner birds (*Buphagus* spp.) and they, like other cleaners, remove and consume ectoparasites and other detrimental material from the bodies of another species, termed a client (Feder 1966), benefiting the clients health (Clague et al. 2011b). As well as a nutritional benefit, cleaners also gain predatory protection (Cheney et al. 2008), which explains the first documented observation of cleaning in the fifth century BC, being between a small hummingbird (*Throchilus* spp.) and a Nile crocodile (*Crocodylus niloticus*, see Vaughan et al. 2017). Unsurprisingly, cleaning is hence considered to be one of the most remarkable types of ecological interactions observed between taxonomically unrelated individuals (Poulin and Grutter 1996). But how did this extraordinary interaction evolve in the first place?

Cleaner species, like other mutualists, can either be dedicated or facultative. Dedicated species (formerly termed obligate cleaners), rely solely on client gleaned food throughout their life, whilst facultative species are not solely dependent on cleaning for nutrition, with many such species only cleaning when juveniles (Vaughan et al. 2017). Within their environment, cleaners are not abundant themselves, and few species act as cleaners (Grutter et al. 2003; Floeter et al. 2007; Sazima 2011). However, more species act as facultative rather than dedicated cleaners (Sazima 2011; Vaughan et al. 2017) and species vary in the degree of reliance on cleaning. Some facultative cleaners are considered to be just as important in the community as dedicated cleaners, cleaning just as often, and interacting with as many clients (Sazima et al. 2010; Quimbayo et al. 2018), whilst others adopt more opportunistic and/or temporary behaviours (Sazima 2011; Vaughan et al. 2017). Given the benefits of cleaning, what factors limit and regulate the pervasiveness of this behaviour? And why do these part-time mutualistic cleaning strategies exist in the first place?

Although cleaners gain a food resource through removing ectoparasites from clients, they could benefit more if they exploit or abandon their partners (Sachs and Simms 2006). Cleaners can increase their foraging gains by biting higher calorific material from hosts bodies (Eckes et al. 2015) in a behaviour termed cheating. Cheating is detrimental to the hosts health (Poulin and Vickery 1995) and thus cleaning can easily shift from being mutualistic to parasitic (Weeks 2000; Cheney and Côté 2005). Despite this exploitation, mutualisms often remain beneficial across time and space (Frederickson 2017) and cleaners do alter their mutualistic versus parasitic cleaning behaviour to ensure the mutualism is still maintained (Bshary 2002a; Binning et al. 2017b). Clients can help regulate cleaning outcomes by punishing parasitic cleaners through dislodgment or by not re-visiting the same cleaner (Bshary and Schäffer 2002; Sazima 2011) but this is not always the case (Weeks 2000; Soares et al. 2008b; Found 2017). Given that cleaners could gain greater foraging benefits from not cleaning at all, this ultimately raises the question, why are these cleaning mutualisms so ubiquitously maintained?

The occurrence and dynamics of cleaning are, like all other mutualisms, context-dependent (Chamberlain et al. 2014). This is unsurprising given that cleaning occurs in complex and diverse environments (e.g. coral reefs) and as a result, cleaners can interact with a number of different species (e.g. bluestreak wrasse, up to 132 different species, Grutter and Poulin 1998b, oxpecker, up to 12 species, Mooring and Mundy 1996; Koenig 1997). Different partners differ in the quality (Grutter 1994; Arnal et al. 2001; Eckes et al. 2015) and quantity (Grutter 1994; Poulin and Rohde 1997; Soares et al. 2008a; Patterson and Ruckstuhl 2013) of material they host or trade both across and within species (Crofton 1971). Since cleaning essentially involves two individuals interacting at any one time point, the behaviours and traits of one partner will directly influence the behaviours and traits of the other (Wolf and Weissing 2012). Thus, asymmetric partners will lead to asymmetric interaction outcomes. Indeed, cleaning patterns for a given cleaner (e.g. *Elacatinus* spp.) are well documented to vary temporally (Sazima et al. 2000; Côté and Molloy 2003) and spatially (Sikkel et al. 2000; Whiteman and Côté 2002b; Cheney and Côté 2005). It is thus unsurprising that we do not fully understand how cleaning interactions are affected by the complex communities within which they occur: are certain client species, or other biotic variables consistently important in regulating cleaning patterns tempo-spatially? And what are the combined consequences of within and between-species diversity (of both the cleaner and client), on the outcomes of cleaning interactions?

CLEANER-CLIENT INTERACTIONS IN THE CARIBBEAN

Cleaning interactions occur in both terrestrial and aquatic environments, but they are most ubiquitously observed on tropical coral reefs worldwide (Poulin and Grutter 1996; Grutter 2002; Vaughan et al. 2017). Although the bluestreak cleaner wrasse is the most widely studied cleaner fish, these cleaners are not observed in Caribbean regions. Instead cleaning gobies (*Elacatinus* spp. formally *Gobiosoma* spp.) are the predominant dedicated cleaners on these reefs (Côté and Soares 2011). Despite evolving independently, cleaner wrasse and gobies do bear some striking resemblances: both are small in size and express light blue and black colouring, which likely signals their identity as cleaners (Stummer et al. 2004; Cheney et al. 2009). They also both occupy topological reef features, known as cleaning stations (Potts 1973a) where they wait for clients to visit them (Potts 1973a; Côté et al. 1998; Mills and Côté 2010). However, the dynamics of their interactions are somewhat different: cleaning gobies patterns are simpler than bluestreak wrasse. Gobies do not show obvious advertising dances to attract clients, manipulate their clients through tactile stimulation (Côté and Soares 2011), where the cleaner uses their pectoral fins to stroke the clients' body (Bshary and Würth 2001), and perhaps more relevant here, do not obviously adopt their cleaning behaviour to clean certain clients more favourably to encourage their return (Grutter and Bshary 2003). Thus, goby cleaning behaviour will likely reflect true decisions to clean certain clients. Therefore, Caribbean cleaning interactions represent a model system for investigating what factors can influence the dynamics and occurrence of cleaning.

Cleaning gobies range extends across the Caribbean, and Cardiff University is fortunate enough to have a long-term (8+ year) field site in Tobago. From 2010 – 2017 data has been collected on the cleaning interactions between sharknose gobies (*Elacatinus evelynae*) and their clients occupying the same cleaning stations on the same reef (Booby Reef) in the Man O' War Bay, situated in the north of the island. This data set includes a record of client species identity, cleaner and client behaviour, and presence and abundance of cleaner and client species at both the cleaning stations and on the reef. Ultimately these data provide a unique opportunity to examine cleaning patterns over time and space, and thus forms the main basis of work presented here.

THESIS AIMS

The objective of this work was to investigate what factors influence the dynamics and hence occurrence of cleaning interactions across individuals, populations and species. To help our understanding of how mutualisms evolved, **Chapter 2** presents an across phyla review on the ubiquity of cleaning and discusses factors that drive variations in the presence of this behaviour. This review takes a novel approach by considering cleaning in tandem with the parallel behaviour observed between conspecifics – allogrooming. What maintains the occurrence of cleaning is also considered in **Chapters 3** and **4**. **Chapter 3** considers why part-time cleaning strategies can exist, by investigating the importance of the interaction to a facultative cleaner, juvenile blue-headed wrasse (*Thalassoma bifasciatum*), whilst **Chapter 4** investigates cleaning behaviour stability of an iconic dedicated cleaner, the bluestreak wrasse (*Labroides dimidiatus*) under naturally stressed conditions. This study was carried out in the Great Barrier Reef, since this reef ecosystem is, and has been, experiencing an unprecedented level of anthropogenic induced stress (De'ath et al. 2012; Hughes et al. 2017). To determine how cleaning is affected by the complex communities within which they occur, the remaining four chapters investigate the cleaning behaviour of the predominant Caribbean cleaner, the sharknose goby (*Elacatinus evelynae*) on Booby Reef, Tobago. Using an 8 year long-term data set, **Chapter 5** first quantifies how consistent cleaning patterns are over time on the same reef. This chapter specifically determines whether different client species within the community are cleaned consistently each year. Following on from this, **Chapter 6** identifies important biotic factors, linking to the reef community, that can consistently influence both cleaner and client behavioural patterns across time. Cleaner and client behaviour feedback with one another, so individual level behaviours of both parties will ultimately influence patterns observed at a population level (Wolf and Weissing 2012). Thus **Chapters 7** and **8**, investigate cleaning patterns at an individual level from both the cleaners, and clients' perspective. **Chapter 7** specifically asks whether individual differences in the cleaners' behaviour influences who interacts with whom and how. **Chapter 8** shows how photo-identification can provide a novel tool for studying individual reef fish over time and applies this method to determine whether there are between-individual differences in client behaviour at different cleaning stations. Each chapter is presented in a self-contained manner but together they aim to contribute to our dynamic knowledge of cleaner-client mutualisms.

Chapter 2

The Cleaning-Grooming Continuum: From Specialists to Generalists

A shortened version of this chapter is in preparation for journal submission.

ABSTRACT

Tactile grooming between conspecifics (allogrooming) and heterospecifics (cleaning) are iconic, charismatic behaviours, used for removing parasites from another individual. Given that parasites are the most common form of life on Earth, why do many species not express inter- and/or intra-specific grooming behaviours, and why do others express these behaviours frequently? By considering both conspecific and heterospecific grooming together, this chapter presents a novel perspective on why and how this behaviour evolved. Here, this chapter considers these interactions within a proposed three-dimensional continuum, which together considers the interaction outcome (mutualistic to parasitic), interaction reliance (full-time to rare) and interaction type (intra- to inter-specific grooming). Data mining of primary literature reveals that despite evolving multiple times across three phyla, intra- and inter-specific grooming is currently only documented in 1.89% of all animal families. Intra-specific grooming mostly occurs in terrestrial environments, while inter-specific interactions predominantly occur in aquatic environments. Nevertheless, intra- rather than inter-specific interactions are more common, since the latter represents a specialist foraging strategy whilst intra-specific grooming is not density limited, instead serving a strong social bonding role. This chapter also discusses genetic, behavioural and environmental factors that could influence the occurrence of inter- and intra-specific grooming and concludes that there are multiple predictive factors: intra-specific groomers are adapted for removing small material from other bodies and are gregarious species which form strong, generally kin-based social structures, whilst inter-specific groomers tend to be small animals living in spatially complex and heterogeneous environments that can regulate their own interactions with clients through opportunistic behaviours or behavioural manipulations. These factors ultimately influence the dynamics of the interaction, and this work shows that different

species, families and classes occupy a sliding dynamic position along the grooming-cleaning continuum.

INTRODUCTION

Nearly every animal on the planet is directly or indirectly involved in a mutualistic interaction (Bronstein 2015), yet the mechanisms underlying the development of these beneficial interactions between species is still not clear. Studies on the evolution of mutualisms often solely focus on the mutualism itself, but why not consider their occurrence in tandem with an equivalent behaviour observed between conspecifics? Cleaning for example, is an iconic mutualism, and involves an individual removing parasites and material from the body of a heterospecific (Feder 1966). Cleaners gain nutrition by eating gleaned material whilst the individual cleaned should benefit from ectoparasite removal (Clague et al. 2011b). Allogrooming (or allopreening for birds) is also essentially a cleaning behaviour, with one individual removing parasites and other material from the body of a conspecific (Poulin and Grutter 1996). Despite being very similar interactions, allogrooming and cleaning, have, for the most part, been considered as mutually exclusive, even though species have been documented to "inter-specifically allopreen" (i.e. clean, Verbeek et al. 1981), and/or "alloclean"/ "intra-specifically clean" (i.e. allogroom, Sulak 1975; Fujishima and Wada 2013). Rather than considering cleaning and allogrooming as discrete interactions, this chapter suggests that these behaviours form a grooming continuum, ranging from those individuals that only remove material from conspecifics to those that solely clean heterospecifics.

Given the potential benefits of cleaning/grooming, and that over 50% of all living animals are parasites (Price 1980; Poulin and Morand 2000), these behaviour should be widespread across taxa. The benefits of cleaning/grooming however, are not always symmetric, with even slight changes in the environment or behaviour of one partner shifting the outcome of the interaction along a continuum. At one end of the spectrum, both parties benefit (mutualism), whilst at the other, one partner benefits and the other is harmed (parasitism, Holland and DeAngelis 2010; Brown et al. 2012). Even within the same environment, different interaction strategies can be observed. For cleaning, for example, some species can be full-time dedicated cleaners for part (often as juveniles) or all of their lifespan, gaining all their nutrition from client-derived material (Poulin and Grutter 1996), whilst other species may be part-time facultative cleaners, not solely dependent on cleaning for nutrition at any one time (Vaughan et al. 2017). The reliance on tactile grooming between conspecifics and heterospecifics can thus also be considered along a continuum, ranging from individuals which frequently clean/groom, to those which rarely clean/groom.

This review considers the costs and benefits of cleaning and allogrooming (hereafter termed inter- and intra-specific grooming respectively), in the context of three continua: interaction outcome (mutualistic to parasitic), interaction reliance/frequency (full-time to rare) and interaction type (intra- to inter-specific grooming) (Figure 2.1), to further knowledge on the evolution and maintenance of these different grooming strategies (Brown et al. 2012). To fully understand why behaviours exist, their phylogeny, causation, development and function should be considered (Tinbergen 1963; Bateson and Laland 2013), thus this review asks: how pervasive is intra- and inter-specific grooming across animal taxa? And what factors drive this variation in behaviour?

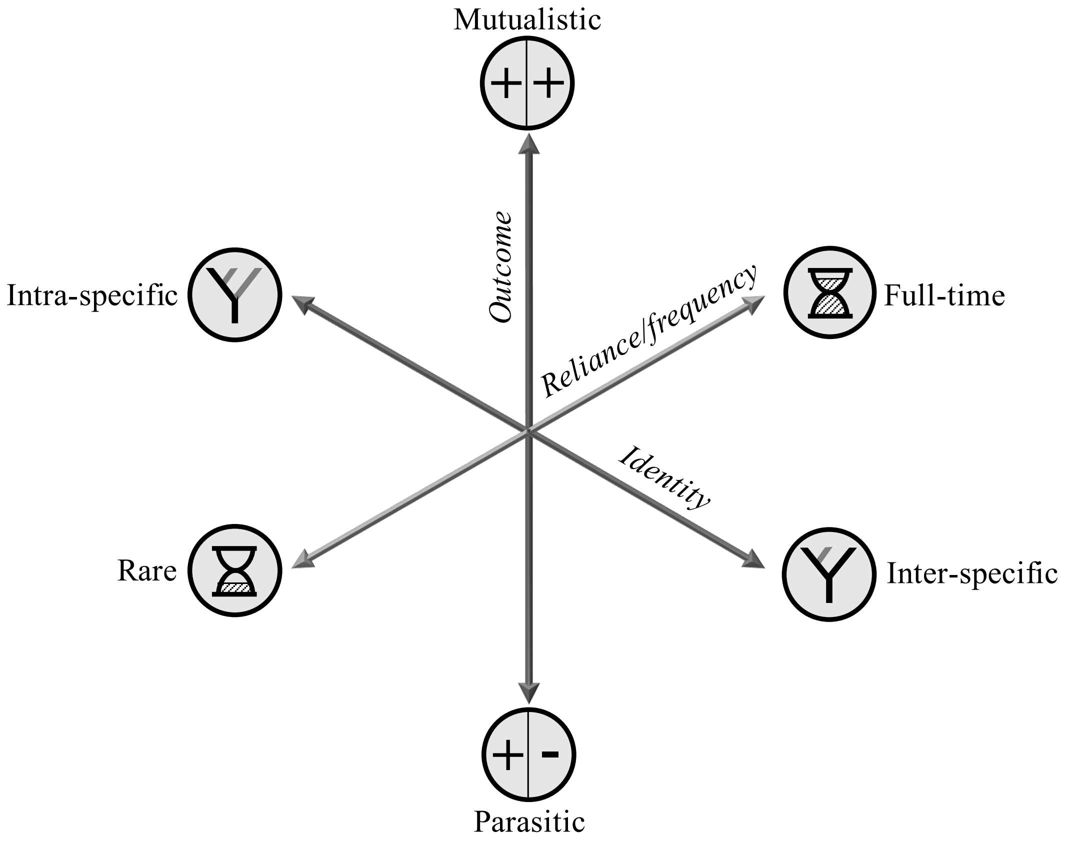


Figure 2.1: The cleaning-grooming continuum presented as a set of three axes. Between individual grooming can occur inter- and/or intra-specifically, and their occurrence can differ from those that groom regularly to those that only groom rarely. Finally, during any of these interactions the benefits to each party may be symmetric or asymmetric meaning that the interaction outcome can range from mutualism to parasitism.

METHODS

Web of Science was data-mined using the search terms: allogroom*, allopreen* and clean* (up to: August 2017). Due to the large volume of results produced by clean* (57,538) this search was refined to exclude non-biological, non-primary literature results using the Web of Science categories and research areas tool (producing 10,516 results). Studies were only included here if intra- and/or inter-specific grooming behaviours were witnessed during the course of a particular study. For some inter-specific groomers, species (notably fish) have been assumed to be groomers based on the detection of ectoparasites in their stomach gut contents (e.g. Randall 1967). Many ectoparasites, however, are highly mobile (Grutter 2003), detach easily and frequently from their hosts (Grutter 1995b; Chambers and Sikkel 2002), and can be preyed upon while in the environment. Thus, these studies were not included here as grooming may not necessarily occur for these species. Similarly, the interaction between crayfish and branchiobdellid worms was also excluded. Although this has previously been considered as inter-specific grooming (Brown et al. 2012), there are no reports of the worms actively removing material from crayfish. The interaction is assumed to be grooming by the location of the branchiobdellids on the host (James et al. 2015) and the positive fitness benefits to the host (Brown et al. 2012). However, other organisms living externally on an animal can be mutualists (Ross 1983; Puce et al. 2008) but are not considered groomers.

The type of interaction (intra- and/or inter-specific grooming), the grooming animal family, and the family(s) of the grooming receivers were recorded from data-mined studies. It was also noted whether the behaviour was observed in captive or wild conditions, as captive conditions can promote unnatural behaviours (Volpato et al. 2009). Anecdotal observations (Abe et al. 2012) and interactions reported in the grey literature and media (e.g. crabs cleaning marine iguanas in Planet Earth 2 2017, and mongooses cleaning warthogs reported by Wildlife Conservation Society 2016) that were not also covered within the primary literature, were excluded from the current study based on lack of scientific validation. Studies which did not report the frequency of the observed interactions were also excluded (like Sazima and Sazima 2010) as it was not clear whether these were isolated observations.

THE PERVASIVENESS OF INTRA- AND INTER-SPECIFIC GROOMING ACROSS ANIMAL TAXA: PHYLOGENY

Grooming is currently observed in three of the 36 animal phyla: Arthropoda, Echinodermata and Chordata. Grooming animals thus range from small insects and brittle stars to large, cognitively advanced primates (Figure 2.2). Despite being spread across three different phyla, inter- and intra-specific grooming behaviours are relatively rare, only being clearly documented in 1.89% of all animal families (100 grooming families from a total of 5300 animal families, Mora et al. 2011). Nevertheless, within the seven classes (Figure 2.2) where grooming is observed, the prevalence of the behaviour ranges from 1% of Insecta families (and is restricted to colonial living eusocial insects) to 23% of Mammalia families (Figure 2.2). The function of the behaviour differs between these animals, likely driving the differences in grooming presence across classes. For example, intra-specific grooming serves a hygienic, disease control function in the high density living, eusocial insects (Konrad et al. 2012; Bąk and Wilde 2015; Theis et al. 2015), which only make up a small proportion of the Class Insecta (three eusocial orders out of 30 Insecta orders). Contrastingly, intra-specific grooming in Mammalia primarily serves a social function (Di Bitetti 1997; Kutsukake and Clutton-Brock 2006), which largely drives their mating systems (Clutton-Brock 1989).

Intra-specific grooming is more pervasive than inter-specific grooming: out of the 98 grooming families, 59% families intra-specifically groom (found within Insecta, Mammalia and some Aves), whilst 29% groom inter-specifically (within Malacostraca, Ophiuroidea and Reptilia, and most grooming Actinopterygii). The remaining 12% of families (within Actinopterygii and Aves) express both grooming strategies (Figure 2.2). As it stands, intra-specific grooming occurs predominantly in terrestrial habitats, whilst inter-specific grooming dominates aquatic environments. Whether these patterns reflect real biological differences or sampling bias is unknown. Across 502 studies, 27% focussed on inter-specific interactions among fish (especially the Labridae family), whilst mammals were a major focus of the intra-specific grooming literature (43% of all studies, especially Cercopithecidae family). In contrast, only one study documented grooming for Reptilia (Krawchuk et al. 1997). The mismatch between the intra- versus inter-specific grooming fields was also apparent, especially for fish, where documented cases of intra-specific grooming were predominantly obtained from searching through groomers' client lists, rather than specifically referred to. Environmental bias will also reflect our limited exploration of marine environments (currently one to two thirds of marine diversity has been undescribed (Appeltans et al. 2012) and there is presently no clear confirmation that inter-specific grooming regularly occurs between terrestrial and aquatic individuals; apart from an anecdotal observation (and therefore not included in the current data analysis) of albatross picking material off the body of sunfish (Abe et al. 2012).

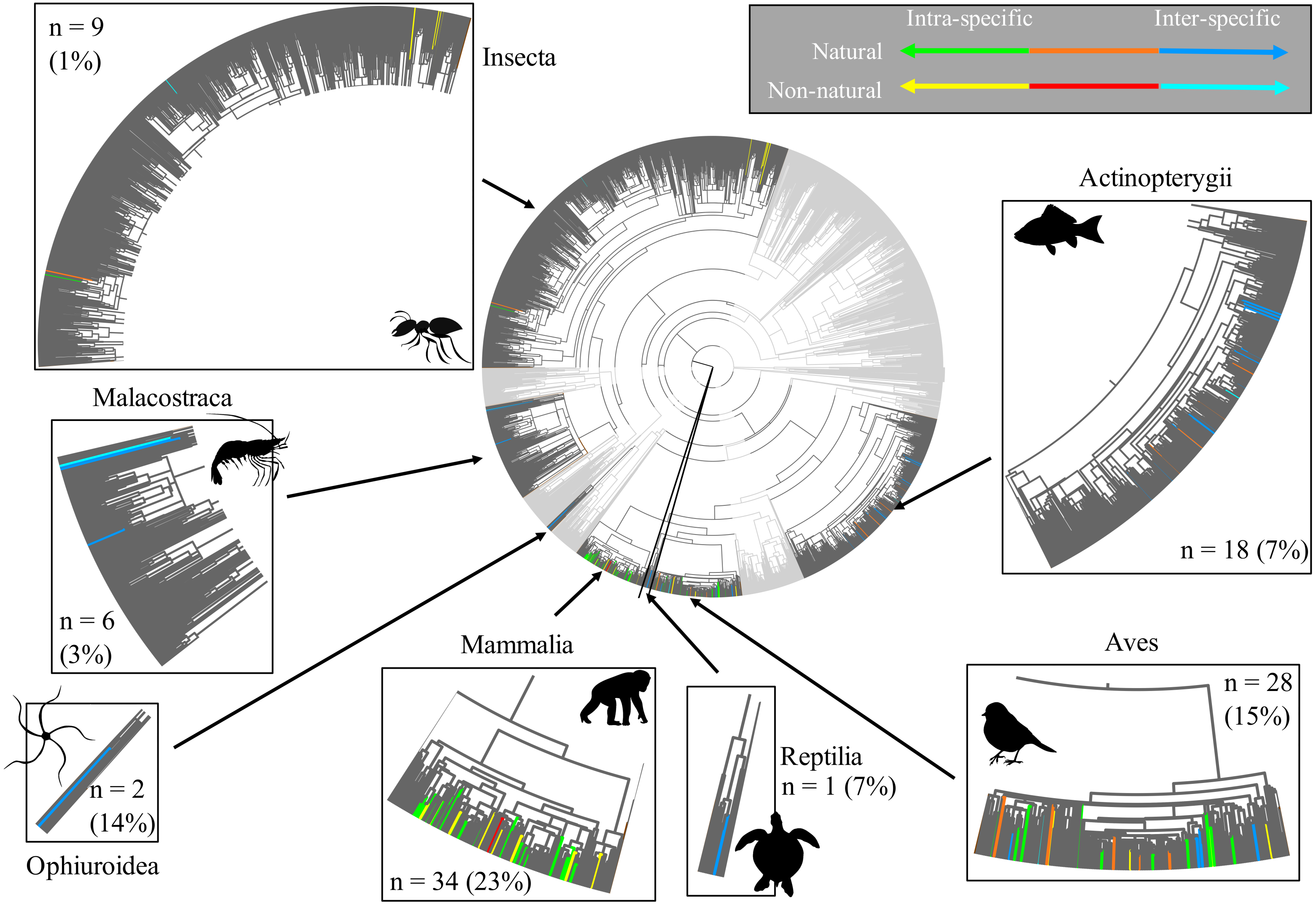

n = 9
(1%)
Insecta
Intra-specific
Inter-specific
Natural
Non-natural
Actinopterygii
n = 18 (7%)
Malacostraca
n = 6
(3%)
n = 2
(14%)
Ophiuroidea
Mammalia
n = 34 (23%)
Reptilia
n = 1 (7%)
Aves
n = 28
(15%)

Figure 2.2: Occurrence of intra- and inter-specific grooming across the animal kingdom. Phylogenetic tree represented at a family level with lighter sections showing classes where grooming is not observed. Coloured lines show the occurrence of grooming within each family, with colours representing the nature of the interaction: green/yellow lines show families where only intra-specific interactions are observed, whilst blue lines represent families which only inter-specifically groom. Orange and red represent families where both intra- and inter-specific grooming is observed in natural (orange) or captive (red) settings. Numbers represent the number of families where grooming is observed within each animal class, with percentages showing the proportion of families within each class that engage in grooming.

FACTORS INFLUENCING THE PERVASIVENESS OF INTRA- AND INTER-SPECIFIC GROOMING ACROSS ANIMAL TAXA: CAUSATION, DEVELOPMENT AND FUNCTION

To some extent, all grooming is mediated by parasites: the removal of parasites and other unwanted, and potentially harmful, material from either a conspecific or heterospecifics body, can be a primary or secondary function of grooming. Thus, the occurrence of grooming will be influenced, to some degree, by the ecology and biogeography of the parasite community.

Parasite communities

Intra- and inter-specific grooming usually occurs in environments with warmer climates: fish inter-specific grooming is ubiquitously observed on tropical coral reefs for example (White et al. 2007). Within tropical environments, parasite assemblages are larger and more diverse compared to the poles (Guernier et al. 2004; Bordes and Morand 2009), and the global occurrence of parasites and the spread of disease is closely linked with climate (Guernier et al. 2004). Tropical environments are also more stable in their seasons compared to more temperate environments and therefore the parasite community will be more reliable for both inter- and intra-specific groomers (Hawley and Altizer 2011). For inter-specific groomers, grooming serves a food acquisition function. The occurrence of those groomers which gain all their nutrition from client-derived material, adopting a specialist foraging behaviour (Bridcut and Giller 1995; Amundsen et al. 1996), will thus be limited within an environment by parasite availability; the benefits of food acquisition via grooming will only be tangible if competition for resources is low (Stephens and Krebs 1986). Investigating the occurrence of more facultative grooming species may provide insight into how this inter-specific interaction developed. Facultative part-time groomers adopt generalised feeding behaviours, and it is likely that their grooming behaviour developed opportunistically (Sazima 2011) as learnt extensions of existing food acquisition behaviours. For example, facultative grooming fish commonly feed on benthic invertebrates (Poulin and Grutter 1996) and both birds and fish that groom part-time can already associate with other mammal/reptile/fish species for foraging gains (Sazima et al. 2004; Tomazzoni et al. 2005; Sazima and Sazima 2010; Sazima 2011; Thomson et al. 2015; Ritter and Amin 2016). These associations would thus provide opportunities for potential groomers to interact with potential clients.

The occurrence of parasites within an environment may be limited by the availability of vectors (Guernier et al. 2004). Grooming tends to occur in environments where contact between individuals is high. For example, communal/gregarious species engage in grooming (e.g. eusocial honey bees, *Apis* spp., see Bąk and Wilde 2015; partner buff-breasted wrens, *Cantorchilus leucotis,* see Gill 2012; and chimpanzee troops, *Pan troglodytes*, see Newton-Fisher and Lee 2011) and coral reefs are biodiversity hot spots (Hughes et al. 2002). Parasite diversity and abundance will increase with increasing species diversity and abundance within a taxon, providing high evolutionary pressure for parasite removal methods. Ultimately this will provide more food resources for inter-specific groomers and increase the fitness costs for host species and their social group. Thus, intra or inter-specific grooming may be more likely to occur in smaller more isolated environments where the frequency of between species or within species interactions is high.

The diversity and identity of parasites involved in grooming is often overlooked when investigating grooming dynamics. Parasite species are likely to influence the pervasiveness and function of both inter- and intra-specific grooming. Gnathiid and copepod crustaceans are the most cited ectoparasites for fish and shrimp grooming (Cheney and Côté 2005; Côté and Soares 2011) but monogeneans and other ectoparasites are also likely to be involved (Grutter 1994;1995b;2002; de Souza et al. 2014). These other parasite species are rarely considered (despite their high abundance), probably because of their small size and likelihood of being lost unless the host is carefully handled. The type of food/parasite consumed (and associated nutritional value) from the host probably influences the behaviour of both dedicated and part-time grooming fish (Eckes et al. 2015).

Susceptibility to parasites

When interacting with a parasitised individual there is a risk of infection to the groomer. The extent of this risk will depend upon the groomers' resistance, grooming frequency and/or the type of interaction. An individual's susceptibility to parasites is fundamentally driven by their immunity (Hawley and Altizer 2011), thus resistant individuals may be more able to groom others at a lower infection risk to themselves. This may certainly apply to dedicated inter-specific groomers, since individuals constantly exposed to certain parasites across generations may be more resistant to infection (Fefferman et al. 2006); exposure to parasites influences an individual's immune response (Chaplin 2010). Thus dedicated inter-specific groomers, which can engage in a high number of grooming interactions with a diverse range of clients daily (e.g. *Labroides dimidiatus* interact with an average of 2297 fish per day, Grutter 1996a; Figure 2.3), may have few parasites themselves and immune systems specifically adapted to evade the parasites they commonly encounter on their clients. This hypothesis has not yet been tested, but parasites are evident on facultative inter-specific groomers (Hobson 1971; Treasurer 1997; Bron and Treasurer 2009), and non-grooming shrimps spend a considerable proportion of their day self-grooming (a behaviour not reported for grooming shrimps, Bauer 1978;2004;2013). Dedicated inter-specific groomers are also rarely observed/reported to visit other groomers (both con- and heterospecifics) whilst groomers that only clean part-time also groom conspecifics (Hobson 1971; Sulak 1975; Hobson 1976; Sazima et al. 1999) or attempt to elicit grooming from heterospecifics (**Chapter 3**; Arnal et al. 2000; Francini–Filho et al. 2000; Zander and Sotje 2002; Krajewski 2007). Despite not necessarily being directly affected themselves by parasites, dedicated inter-specific groomers may act as vectors of parasites or reservoir hosts by consuming infective stages (Lu et al. 2000; Jones et al. 2004).

Species differ in their innate versus adaptive immune responses, with ‘slow living’ species (low reproductive rates and slow development) hypothesised to rely more on adaptive immune responses to new infection compared to ‘fast-living’ species (Lee 2006). These differences may explain why parasite removal serves a more secondary function for mammalian groomers versus a hygiene regulation function in the short-lived eusocial insects where their immune system cannot cope with parasite exposure to the same degree. A strong immune system comes with a high energetic demand (Sandland and Minchella 2003), and thus gaining food from grooming may be a costly and sub-optimal strategy. Indeed, individual parasite consumption is of low calorific value (White et al. 2007; Eckes et al. 2015, although other nutritional gains are not clear) and this may explain why dedicated groomers often consume other, more calorific material from their clients as well (e.g. mucus, Eckes et al. 2015, and blood, Weeks 2000). Nevertheless, the immune system may play a key role in the persistence of grooming behaviour and deserves further attention.

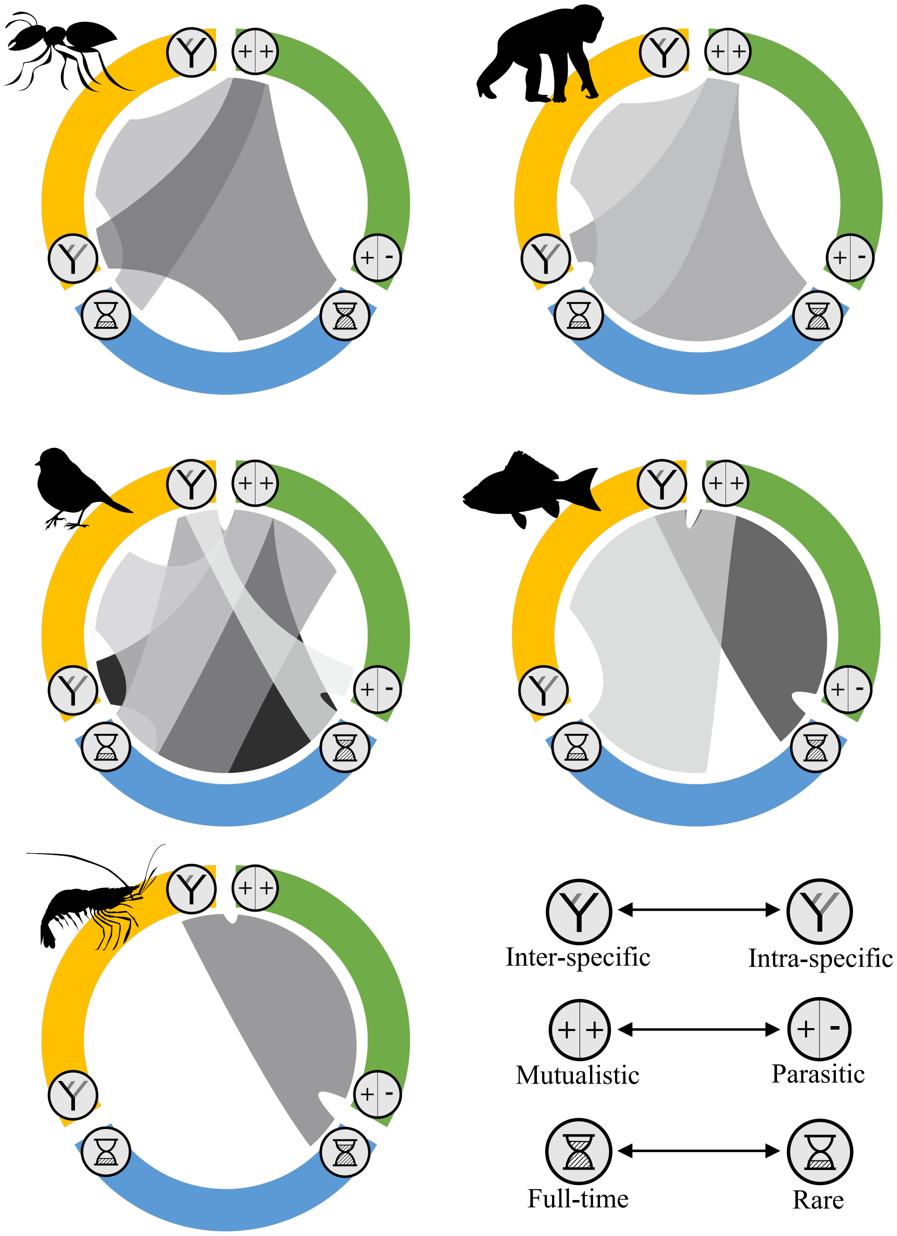


Figure 2.3: Estimates of where five different animal classes (Insecta, Mammalia, Aves, Actinopterygii and Malacostraca) lie along the proposed three grooming-cleaning continua. Individuals, species and families within these classes cannot be absolutely placed along the three continua due to the dynamic and context-dependent nature of intra- and inter-specific grooming. Shaded regions represent the links between three continua: grooming identity (intra- versus inter-specific grooming with middle section representing both), grooming outcome (ranges from parasitic to mutualistic) and reliance/frequency (ranging from rare to full-time). Across all classes, grooming dynamics vary along at least one of the three axes and is dependent upon relative positions on the other continua (represented by different greys), and a suite of factors that govern the persistence of grooming (see text). Reptilia and Ophiuroidea are not presented here as not enough information is available on their grooming dynamics (Figure 2.2).

Parasites pose less of a risk to a groomer when the species is more taxonomically distant to their client, although this will depend upon the generalist nature of the parasite (Woolhouse et al. 2005). Grooming birds, which interact with mammals (e.g. oxpeckers, *Buphagus africanus* and *B. erythrorhynchus*), may thus be less susceptible to infection. In contrast, among intra-specific social groups, where there is a high population density and low genetic variation, parasite susceptibility and spread is high (such as in eusocial insects, Cremer et al. 2007). Eusocial insects, which intra-specifically groom for personal hygiene and to increase their 'social immunity' (Konrad et al. 2012; Okuno et al. 2012), are at high risk from infection and thus also use other physiological infection control methods, such as venom (Tranter and Hughes 2015), chemical disinfection (Bulmer et al. 2012; Tragust et al. 2013) and upregulation of immune genes (Liu et al. 2015) to reduce infection risk during grooming and epidemics within colonies. The external body type of a host may also influence the occurrence of grooming, since skin surfaces can influence the prevalence and transmission of parasites (Gans and Baic 1977; Arnold 1986; Watson et al. 2015). Body hair, for example, is a common trait of intra-specific mammalian groomers; hairlessness is thought to have evolved in response to the ecological pressures of parasite transmission (Pagel and Bodmer 2003; Thompson 2010)

The need for parasite control

Good hygiene promotes good fitness, and many species can control their hygiene and ectoparasite abundance through their own behaviours. Given that self-hygiene will regulate the abundance and diversity of parasites, the extent of an individual's hygiene will influence the occurrence of inter- and intra-specific grooming strategies. Weaver ants (*Oecophylla* spp.), for example, which excrete and spread venom across their bodies, groom conspecifics less frequently than non-venom producing ants (Tranter and Hughes 2015, Figure 2.3). Autogrooming or self-preening/grooming is an innate behaviour for removing unwanted material (Spruijt et al. 1992), and is observed in many eusocial insects (Theis et al. 2015), bird species (Radford 2012) and mammals (Mooring et al. 2004). Self-grooming can however, result in hyper-infections (Csata et al. 2014), as can intra-specific grooming (Theis et al. 2015). To some extent, intra-specific grooming is thought to have evolved as an additional strategy for removing parasites from areas of the body that are difficult to reach during self-grooming (Mooring 1989), and groomers often target inaccessible body regions such as the head, neck and back (Borries 1992; Reichard and Sommer 1994).

In contrast, inter-specific grooming has primarily evolved in species which are morphologically constrained to self-groom and/or where intra-specific grooming rarely occurs, such as fish (Figures 2.2 and 2.3; Losey 1987; Hart 1990; Poulin and Grutter 1996) and many ungulate species (common 'cleaner bird' clients, Sazima 2011). For these species and many amphibious, non-social insects, reptilian or sessile species, for which inter- and intra-specific grooming is not ubiquitous, these animals use other behavioural methods to control parasite infection. Substrate flashing (Wyman and Walterswyman 1985; Wisenden et al. 2009, although this may too ultimately increase exposure to potential ectoparasites and secondary infection risk), avoidance behaviours (Barber et al. 2000; Wisenden et al. 2009; Mikheev et al. 2013), group formation/increased social contact (Poulin and Fitzgerald 1989; Barber et al. 2000; Reynolds et al. 2018) and behavioural fever (Elliot et al. 2002; Richards-Zawacki 2010; Mohammed et al. 2016), all act as preventative parasite control methods, and thus inter-specific grooming may have developed to exploit the need for a removal method once infected.

Animal behaviour plays a key role in mediating disease transmission (Hawley and Altizer 2011), and thus parasites alone may not be the only factor influencing the frequency of grooming. Indeed, for clients visiting inter-specific grooming fish/shrimp, both groomer and client can initiate the interaction (see "Signal to initiate"), and it is not clear whether the client's parasite loads are driving their willingness to engage in grooming: there is limited evidence that clients clearly benefit through parasite removal or a stress reducing tactile reward from the interaction (Soares et al. 2011). Long-term studies have, however, begun to show some minor fitness implications to the client (Waldie et al. 2011). Thus, here this chapter now discusses additional traits (physical and behavioural) which may influence the pervasiveness of intra- and inter-specific grooming across animal taxa.

Physical – Body size

From an ecological and evolutionary stand point, body size is one of the most important attributes of an organism (Werner and Gilliam 1984), and appears to influence the frequency of both intra- and inter-specific grooming. Within species, body size influences who grooms who (e.g. dominance position) and for how long

(Dunbar 1991; Mooring et al. 2000), whilst across species, intra-specific grooming is not observed in very large social (see "Social structure") species which have high rates of other tactile behaviours between conspecifics (Karenina et al. 2010; Makecha et al. 2012) such as elephants (Eliphantidae) and sperm whales (*Physeter microcephalus*). Although body size can correlate with parasite loads (Poulin and Rohde 1997; Grutter and Poulin 1998a), here instead the size of the parasites in relation to the host may influence grooming occurrence. For larger species, the presence of small external parasites may pose less of a cost (e.g. ectoparasites can reduce movement efficiency, Binning et al. 2017a), than a small species infected with a small external parasite. For example, respective relative body sizes for a whale louse (*Cyamus boopis*) infecting a male humpback whale (*Megaptera novaenangliae*) are 1:1174 compared to 1:10 for a mite (*Varroa destructor*) infecting a western honey bee (*Apis mellifera*). Thus, smaller species may need more/alternative parasite removal methods to reduce the costs of parasites.

Groomer size is also an important trait influencing inter-specific grooming. Inter-specific groomers are relatively small in relation to their clients, which facilitates the removal of ectoparasites from less accessible areas of the body (including the mouth and gills of fish, Karplus 2014). For others, the success of inter-specific grooming (Sazima and Sazima 2010; Sazima 2011) and frequency of the interaction (Stummer et al. 2004; Cole 2009), decreases as the size of the grooming species increases, reducing the size differentiation between the groomer and client. Thus size will influence which groomers interact with which clients (Sulak 1975). Bigger bodied animals have higher energy demands (Speakman 2005), and thus energy gained from consuming host material may not be sufficient for larger grooming species (Leclercq et al. 2014). Indeed, for the relatively small dedicated cleaner wrasse (e.g. *Labroides dimidiatus*, max. total length 14 cm, Froese and Pauly 2018), fish have been recorded consuming up to 1200 gnathiids daily (Grutter 1996a).

Physical – Body features

The ontogeny and body shape of a grooming species can influence the reliance of a species on grooming. Small mouth gapes and rapid gape cycles, for example, as exhibited by the Labridae and many juvenile fish inter-specific groomers, will assist with ectoparasite removal (Baliga and Mehta 2015), and beaks (e.g. birds), dentition (e.g. Canidae), setae (e.g. Insecta), pincers (e.g. Malacostraca) and phalanges (e.g.

Lorisidae, Radhakrishna and Singh 2002), all help to facilitate the dexterity needed to remove small material from a conspecific or heterospecific.

For the dedicated inter-specific groomers which wait for clients to visit them, their body colouration and patterning can act as an attractant. The conspicuous blue colouration of *Elacatinus* spp. and some Labridae grooming species is an example of convergent evolution (Côté 2000) and likely strongly signals the groomers' identity as it is visually accessible to a wide range of clients against a variety of backgrounds (Stummer et al. 2004). Many dedicated and facultative grooming fish also have yellow body colouration, which strongly contrasts against blue water (Cheney et al. 2009). The black lateral line of some fish groomers may also appease negative behaviours (Alphen 1999). Together, this combination of colours may have evolved to advertise grooming services and reduce predation threat (Lettieri and Streelman 2010). Despite being colour-blind, grooming shrimps also express conspicuous colouration which may also function as an inter-specific signal (Caves et al. 2016). The importance of colour as a trait of dedicated inter-specific groomers can also be highlighted by the evolution of aggressive mimics that do not groom but have evolved to look similar to grooming fish species. While the bluestriped fangblenny (*Plagiotremus rhinorhynchos*) looks morphologically like a juvenile bluestreak wrasse and thus attracts clients, it will subsequently bite these fish (Hansen et al. 2017) whilst also gaining the benefits of predator protection (Cheney 2013). In grooming systems (e.g. 'cleaner birds') where groomers choose clients themselves and interact with non-threatening animals with "impoverished" colour vision (mammals, Jacobs 2009), body colouration is not a necessary pre-requisite for these inter-specific grooming species, and these common grooming species are often monochromatic or drab in colour (Sazima 2011). The role of ultra-violet (Vaughan et al. 2017) or fluorescence, which also serve communicative functions in animals (Hunt et al. 2001; Siebeck 2004; Michiels et al. 2008), has yet to be investigated in grooming contexts.

Behaviour – Social structure

Intra-specific grooming commonly occurs between animals which live in groups or close proximity since it can reduce conflict (Matsuno and Urabe 1999; Lewis et al. 2007) and increase social bonding (Di Bitetti 1997). In captivity, intra-specific grooming can be observed in species which do not naturally groom conspecifics (Prieto and Ryan 1978), suggesting that this behaviour develops as a mechanism for coping with the artificial conditions. Stress can also reduce immunity (Hawley and Altizer 2011), potentially making individuals more susceptible to parasites. Some frequent intra-specific groomers also occasionally groom heterospecifics (e.g. common in birds, Figures 2.2 and 2.3), which likely results from the overlap in social proximity (e.g. mixed species roosting in bats, Ancillotto et al. 2014, mixed species insect colony, Seid and Brown 2009 and mixed species bird flocks, Verbeek et al. 1981). Investigating the development of interactions in species which both inter- and intra-specifically groom (Figures 2.2 and 2.3) will be vital to understanding how the grooming continuum develops (Figure 2.1).

Intra-specific grooming animals are not just gregarious, however (gregarious species do not necessarily groom, Engh et al. 2006); intra-specific grooming species have complex, often kin-based, social structures, which also influences the dynamics of the interaction (Radford and Du Plessis 2006; Newton-Fisher and Lee 2011; Radford 2012). For highly related species, such as eusocial insects (Theis et al. 2015), pair bonding animals (Nelson and Geher 2007; Fairbanks et al. 2014), cooperative breeders (Kutsukake and Clutton-Brock 2006) and colonial (Lewis et al. 2007)/group living animals (Slater et al. 2008), grooming a conspecific provides direct and/or indirect benefits (see “Reciprocity”). This may explain why intra-specific grooming is not pervasively observed in fish for example (Figures 2.2 and 2.3), where there is little evidence of kin-based social relationships (Ward and Hart 2003; Kolm et al. 2005). Interestingly, for those fish species which employ parental care in terms of nest guarding, they do groom their own eggs to enhance their chances of survival (Knouft et al. 2003; Maruska and Peyton 2007), but do not display intra-specific grooming behaviours with any other life stages.

Behaviour – Reciprocity

Reciprocity is a common feature across mammal (Hart and Hart 1992; Fairbanks et al. 2014), bird (Gill 2012) and insect (Okuno et al. 2012) intra-specific grooming. Ultimately reciprocity drives the altruistic nature of grooming with a tit-for-tat strategy, where individuals groom another at an initial cost to themselves (Trivers 1971), which is later beneficially returned or exchanged (Hemelrijk and Ek 1991; Henazi and Barrett 1999). This reciprocity however, is not always symmetric as its’ frequency within species can be influenced by dominance position (Radford and Du Plessis 2006; Newton-Fisher and Lee 2011; Radford 2012), age (Mooring 1989) and host body size (Walker and Hughes 2009); nevertheless it increases both the indirect and direct fitness of the grooming individual (see “Social Structure”). Reciprocity in inter-specific interactions is not so straightforward, since the overall benefits to each party serve differing functions: parasite control and tactile stimulation for the client, and food acquisition for the groomer (Grutter 2001). However, reciprocity does play a role in the dynamics of the interaction for dedicated grooming fish and shrimp, where both the groomers and clients behaviour drive the frequency of the interaction (Côté et al. 1998; Bshary and Schäffer 2002). Contrastingly, grooming birds control the frequency of the inter-specific interaction by choosing and associating with clients themselves (Sazima and Sazima 2010), and so there is no basis for reciprocity. Reciprocity is only stable when there are clear relative benefits and future interaction probabilities (Mesterton-Gibbons and Childress 1996) and so reciprocal altruism occurs between individuals that interact repeatedly and where individuals incur an initial cost (Trivers 1971). Dedicated grooming fish and shrimp rely on clients visiting them at their “cleaning stations”, and thus adopt their mutualistic/parasitic tactics to encourage their re-visitation by feeding on non-preferred food types (see “Cheating”, Bshary and Grutter 2006) and using tactile stimulating behaviours to benefit the client (Bshary and Würth 2001; Soares et al. 2011). Clients allow groomers to access all areas of their body (e.g. groomers enter the mouths of their client, Limbaugh et al. 1961; Ritter and Amin 2016) and pause their own foraging for grooming services (Hobson 1971; Grutter 2002); predatory clients do not consume groomers or even other potential clients at “cleaning stations” in exchange for grooming services (Cheney et al. 2008). The occurrence of reciprocity in inter-specific interactions however will be limited by the interacting species cognitive ability to quickly learn and retain past interactions and outcomes; bluestreak cleaner wrasse (*L. dimidiatus*), are more cognitively advanced than other wrasse species (Salwiczek et al. 2012), which has been linked to their ability to alter their levels of cooperative behaviour towards different clients (Cardoso et al. 2015) and may explain how they can successfully interact with many different client species (bluestreak wrasse regularly interact with 132 different species; Grutter and Poulin 1998b).

Behaviour – Cheating

Inter-specific grooming becomes parasitic, i.e., causes harm, when the groomer removes material from the body of another, such as mucous and live tissue, that negatively impacts host fitness (Poulin and Vickery 1995). Cheating is not observed during intra-specific grooming as these interactions are altruistic (see "Reciprocity", Figure 2.3) and groomers benefit from inclusive fitness (Hamilton 1963). Cheating is a strategy used by dedicated inter-specific groomers (e.g. *Buphagus erythrorhynchus,* see Weeks 2000 and *Labroides dimidiatus,* see Grutter and Bshary 2004, Figure 2.3) to access more favourable food items, but is rare in the smaller goby (*Elacatinus* spp.) groomers, which are thought to prefer consuming parasites rather than client material (Soares et al. 2010). For species that groom part-time, they are expected to cheat less frequently, as cheating would decrease the benefits of grooming in relation to the relative benefits of other food acquisition methods (Freckleton and Côté 2003). Indeed cheating by birds that do not heavily rely on grooming is rare (Sazima 2011), and this parasitic behaviour is not documented for facultative grooming fish, suggesting it is also rarely observed (Figure 2.3). The frequency of cheating in full-time groomers can be regulated by seasonal variation in ectoparasite abundance (Cheney and Côté 2005), but more commonly by the clients' behaviour (Bshary and Noë 2003; Chapuis and Bshary 2009). For grooming fish, where clients choose which individuals to visit, cheating can incur a large cost to the groomer, since it may prevent the client from returning (Bshary and Schäffer 2002). To ensure that these groomers can still benefit by consuming their preferred food source, they use complex tactics to regulate the costs of cheating by adapting their behaviour along the mutualism/parasitism continuum to ensure a continued supply of clients, and hence food source (Figure 2.3, Bshary 2002b; 2002a). In contrast, grooming birds always initiate interactions (Sazima and Sazima 2010) and clients responses are minimal; some clients tolerate cheating behaviour, whilst others may temporally dislodge the bird (Sazima 2011). Thus, there are lower costs for the dedicated grooming bird, the red-billed oxpecker (*Buphagus erythrorhynchus*), to frequently parasitise their mammal clients and gain more from the interaction (Figure 2.3, Weeks 2000). The size difference between inter-specific groomers and their client tends to be larger for birds than fish, and thus a single cheat for a fish client may hold greater implications.

This may explain why cheating in inter-specific fish grooming appears to be a stronger constraint on the interaction dynamics.

Behaviour – association with microhabitat features

For inter-specific grooming species which rely on visiting clients (e.g. shrimp and fish), they associate with topological features of the environment which form "cleaning stations". Clients use these "landmark" features (Kulbicki and Arnal 1999) to solicit grooming (Huebner and Chadwick 2012), and to relocate the more mutualistic favourable groomers (Tebbich et al. 2002, see "Cheating"). The presence of suitable microhabitats can even influence the occurrence of inter-specific grooming within these species; sharknose gobies (*Elacatinus evelynae*) occupying brain coral (*Diploria* spp. and *Monastrea* spp.) "cleaning stations" clean 28 clients species continuously (Whiteman and Côté 2002b), whilst sponge dwelling *E. evelynae* occupying the same reef are rarely observed grooming others (Whiteman and Côté 2004a; White et al. 2007). Some facultative grooming fish also associate with a topological feature (Sulak 1975), but whether or not these features function as specific "cleaning stations" is not clear, as in these more heterogeneous sparser environments, organisms tend to aggregate around habitat structures anyway (García-Charton and Pérez-Ruzafa 2001). In contrast, for those inter-specific groomers which always initiate the interaction (e.g. birds), their grooming behaviour does not appear to be associated strongly with particular habitat features (Hobson 1971; Oates et al. 2010), rather they associate with their clients (i.e. perch on backs, Sazima et al. 2004; Sazima and Sazima 2010).

The idea that "grooming stations" exist for intra-specific groomers has not yet been investigated, but conspecific grooming does tend to occur in roosts/resting/sleeping microhabitats (Radhakrishna and Singh 2002; Fairbanks et al. 2014) where other social events happen, presumably as individuals are already in close proximity. Conspecifics do not appear to associate with microhabitats that provide reduced risks of predation during grooming, even though reduced vigilance behaviour is a cost associated with intra-specific grooming (Cords 1995; Blanchard et al. 2017).

APPLICATIONS

Animal welfare is a global issue (Fraser et al. 2013), with the need to increase and promote the well-being of captive animals, not only for ethical reasons, but also for sustainable farming. Stress and disease are major problems for captive animals (Ashley 2007), and grooming can provide biological control. Inter- and intra-specific grooming can reduce or increase stress and conflict across a spectrum of animals (e.g. fish, Soares et al. 2011, birds, Lewis et al. 2007; Radford 2012, and mammals, Matsuno and Urabe 1999; Aureli and Yates 2010), and can reduce (Theis et al. 2015; Villa et al. 2016) or increase (Weeks 2000; Fefferman et al. 2006; Theis et al. 2015) disease prevalence. By knowing potential factors that influence the pervasiveness and dynamics of grooming across animal taxa, and under what conditions, we can manipulate the captive environment to promote or reduce grooming, to the benefit of animal health. For example, the number of familiar cows present in a field increased intra-specific grooming in domestic cows (Takeda et al. 2000; Gutmann et al. 2015), which has a calming effect (Laister et al. 2011), whilst furnished cages reduced stereotypic intra-specific grooming in lab mice (Marques and Olsson 2007). Stereotypic behaviour in captive situations indicates poor physiological wellbeing (Dawkins 2003). Understanding which factors are more important than others in facilitating grooming could also encourage species which rarely groom *in situ* to beneficially groom *ex situ*. Within the aquaculture industry, ectoparasites are a costly problem for farmed fish (see Costello 2009), and wrasse (Labridae) and lumpsuckers (Cyclopteridae) species which rarely inter-specifically groom *in situ* (Potts 1973b) are deployed to groom infected fish *ex situ*. Altering group numbers, and hence competition, increases grooming efficiency (Groner et al. 2013), and by perhaps manipulating other environmental features (e.g. provide ‘cleaning stations’) or altering the chosen species (e.g. to shrimp, or a species which is morphologically more suitable) may also increase the frequency and benefits of disease control.

CONCLUSIONS

Grooming is relatively rare across all animal families, but the behaviour has independently evolved multiple times across three phyla and is important within seven classes. Grooming behaviours have multiple functions, which include social bonding, parasite control and food acquisition. The function and strength of the fitness benefits influences grooming occurrence. Intra-specific grooming is more common than inter-specific interactions, most likely as the latter represents a specialist foraging strategy, regulated by density dependent food competition. In contrast, intra-specific grooming is socially restricted since it serves a strong social affiliation function. The pervasiveness of grooming, can also, to some extent, be genetically controlled; groomers must be morphologically and physiologically adapted to remove parasites and debris from another and itself, and body size limits the dynamics and frequency of the behaviour, especially for inter-specific groomers. Like most behaviours, the environment also has a strong role in influencing the occurrence and dynamics of grooming, and here this chapter present a whole suite of environmental features which effect the interaction to different degrees depending on the interaction type (intra- versus inter-specific), frequency (full-time versus rare groomers), and outcome (mutualism versus parasitism). Due to this plasticity, it is impossible to rank these features predicting grooming pervasiveness in importance, but generally intra-specific groomers are gregarious animals which form strong, often kin-based, social structures (like in terrestrial environments), whilst inter-specific groomers are small animals, relative to their clients, living in isolated spatially complex and heterogeneous environments (like the aquatic environment). These inter-specific groomers also have the ability to regulate their own interactions with clients through opportunistic behaviours or behavioural manipulations. This work should encourage future research to report instances of intra- and inter-specific grooming in different species to determine the consistently of these conditions in predicting the occurrence of grooming. By considering ‘allogrooming’ and ‘cleaning’ together as the same behaviour, and determining which features influence the maintenance of the behaviour across species; this chapter has shown just how dynamic and context specific grooming is. Ultimately, this helps explain why our current understanding of mutualistic/altruistic patterns is not clear; different species, families and classes cannot be absolutely positioned along the grooming-cleaning continuum (Figure 2.3).

Chapter 3

The Selective Cleaning Behaviour of Juvenile Blue-Headed Wrasse (*Thalassoma bifasciatum*) in the Caribbean

A version of this chapter has been published in Behavioural Processes:

Dunkley, K., Cable, J. and Perkins, S.E., (2018). The selective cleaning behaviour of juvenile blue-headed wrasse (Thalassoma bifasciatum) in the Caribbean. Behavioural Processes, 147,5-12.

ABSTRACT

Through the removal of parasites, dead skin and mucus from the bodies of visiting reef fish (clients), cleaner fish have a significant ecosystem function in the ecology of coral reefs. Cleaners gain nutrition from these interactions and through offering a ‘service’ are afforded protection from predators. Given these benefits, it is unclear why more fish do not engage in cleaning, and why part-time cleaning strategies exist. On coral reefs, dedicated species clean throughout their life, whereas some species are facultative, employing opportunistic and/or temporary cleaning strategies. This chapter investigates the cleaning behaviour of a facultative species to assess the relative importance of this interaction to the cleaner. Using a combination of focal and event sampling from a coral reef in Tobago, this work shows that cleaning is not an essential food source for facultative juvenile blue-headed wrasse (*Thalassoma bifasciatum*), as cleaning rates were unrelated to wrasse foraging rates on the substrate. These wrasse displayed two cleaning strategies: stationary versus wandering cleaning, with cleaning frequencies being highest for stationary cleaners. A specific cleaning location facilitated increased cleaning frequencies, and wrasse cleaning rates decreased as cleaner or client abundance increased. This chapter also compared juvenile blue-headed wrasse cleaning behaviour to a resident dedicated cleaner, the sharknose goby (*Elacatinus evelynae*), and showed that, in comparison, juvenile wrasse clean a narrower client range, predominately cleaning three species of

gregarious free-ranging surgeonfish (*Acanthurus* spp.). The wrasse, however, frequently approached these clients without cleaning, which suggests that their selective cleaning strategy may be driven by the acquisition of a particular parasitic food source. Juvenile blue-headed wrasse are generalist foragers, and may thus be limited in their cleaning behaviour by their nutritional requirements, the availability of a suitable cleaning site, and fish density, which ultimately means that they do not adopt more dedicated cleaning roles within the reef community.

INTRODUCTION

In abundant and species rich environments where competition for food is high, different feeding strategies have developed to spatio-temporally partition the available resources between species (Sale 1977). Generalist feeders are characterised by a diverse diet or consume a broad dietary niche whilst specialists show a preference for specific food types or have a narrower dietary range (Bridcut and Giller 1995; Amundsen et al. 1996). As predicted by foraging theory, specialist feeding strategies develop when there are benefits derived from feeding on specific food types (Stephens and Krebs 1986). However, given the tangible benefits, other species must be limited in their feeding strategies or else they too would be expected to adopt these beneficial specialist strategies.

The abundance and diversity of species supported within coral reefs environments makes them one of the most complex habitats in the world (Reaka-Kudla 1997) and multiple feeding strategies have developed to partition food resources. Most coral reef species are generalist feeders (Sale 1977; Froese and Pauly 2018) but cleaning, a symbiotic interaction ubiquitously observed in coral reef communities (White et al. 2007), represents a specialist feeding strategy where a cleaner removes ectoparasites and other material from the body of a heterospecific, a client (Feder 1966). Dedicated cleaner species (formerly known as obligate cleaners, Vaughan et al. 2017) specialise in cleaning behaviour gaining all their nutrition from client derived material (Poulin and Grutter 1996). Since these cleaners interact with a large diversity of clients on a daily basis, including potential predators, dedicated cleaners are also afforded protection from predators (Potts 1973a; Darcy et al. 1974; Losey 1979; Côté 2000). Within a reef environment, several fish species may act as cleaners (Côté 2000), adopting differing cleaning strategies. Facultative cleaners are not solely dependent on cleaning for nutrition (Itzkowitz 1979; Vaughan et al. 2017), and many such species only clean when juvenile. Although a greater diversity of facultative as opposed to dedicated cleaners is known (Côté 2000), research predominantly focuses on the latter (Côté and Soares 2011): namely the Caribbean cleaning gobies, *Elacatinus* spp. (e.g. Whiteman and Côté 2002b; Côté and Molloy 2003; Soares et al. 2008c; 2008d) and the bluestreak cleaner wrasse, *Labroides dimidiatus* (e.g. Grutter 1995a;1999; Gingins and Bshary 2014; Wilson et al. 2014). The extent to which a facultative cleaner gleans food from clients and the wider environment, varies spatio-temporally and between species (Vaughan et al. 2017). Some more specialised facultative species (e.g. *Pomacanthus paru* and *Thalassoma norohanum*) are considered just as central within the reef community as their dedicated counterparts (Francini-Filho and Sazima 2008; Sazima et al. 2010; Quimbayo et al. 2017) as they clean just as often, interacting with as many client species. Other facultative species exhibit more opportunistic and/or temporary cleaning behaviours, and are considered less specialised in cleaning (Vaughan et al. 2017). Investigating why these facultative cleaners do not adopt more dedicated cleaning strategies, given the benefits of cleaning (nutritional, Poulin and Grutter 1996; and predator protection, Potts 1973a; Darcy et al. 1974; Côté 2000), will further knowledge of why part-time cleaning strategies exist.

Blue-headed wrasse (*Thalassoma bifasciatum*) are a prominent member of the Caribbean reef fauna (Feddern 1965) and only clean when juvenile (Feddern 1965; Cheney and Côté 2003). These facultative cleaners occupy the same reefs as the dedicated sharknose goby cleaners (*Elacatinus evelynae*). Both species are considered principle cleaners in the Caribbean (Michael 1998; Cheney and Côté 2003) but the frequency of observed juvenile blue-headed wrasse cleaning is variable across reefs (Feddern 1965; Darcy et al. 1974; Itzkowitz 1979; Johnson and Ruben 1988; Walsh et al. 2017) and it has been suggested that these cleaners play a minor role on the reef despite being highly abundant (Johnson and Ruben 1988). Although ectoparasites have been recovered from the stomachs of these wrasse cleaners (Randall 1967), their reliance on cleaning as a food source has not been quantified. Itzkowitz (1979) described different cleaning strategies exhibited by juvenile blue-headed wrasse on the same reef in Jamaica; stationary versus wandering cleaners, but the prevalence of these strategies is also unknown. In comparison to other facultative cleaners (e.g. *Thalassoma norohanum*, see Francini–Filho et al. 2000; Quimbayo et al. 2017), these wrasse are considered less specialised in their cleaning, adopting a more opportunistic approach (Itzkowitz 1979; Johnson and Ruben 1988).

Here, this chapter investigated the relative importance of cleaning to juvenile blue-headed wrasse to further knowledge on why part-time cleaning strategies exist. Using the behaviour of the resident dedicated cleaner species (sharknose goby) occupying the same reef in Tobago as a comparison, the cleaning strategies of the facultative wrasse were investigated through behavioural observations. To investigate why these wrasse cleaners do not adopt more specialised cleaning roles within the community, it must be determined what limits their cleaning. Space is the most competitive resource on coral reefs (Sale 1977) and although cleaning represents an opportunistic foraging strategy for juvenile blue-headed wrasse, the prevalence of cleaning strategies of a species can vary spatially (Vaughan et al. 2017), with cleaning stations being an important requisite for other cleaner species (Whiteman and Côté 2002b; Huebner and Chadwick 2012). Thus, it was hypothesised that cleaning by these wrasse will also vary spatially with the frequency of cleaning differing between wandering versus stationary cleaners (like in **Chapter 4**). The prevalence of feeding strategies within a population and between species is also regulated by density dependent competition, as more individuals adopting a strategy, and competing for resources, will reduce the benefits gained by each individual (Krebs 1979). Thus, it was hypothesised that juvenile blue-headed wrasse cleaning would also be limited by the number of wrasse cleaners (increased competition) but would be positively influenced by an increase in the number of clients, reducing competition. The importance of cleaning to a species can be assessed by investigating the cleaners wider diet (Whiteman and Côté 2002b). Juvenile blue-headed wrasse predominantly feed on benthic organisms (Feddern 1965) and so it was hypothesised that if cleaning does provide a compulsory component of their diet, the foraging rate of individual wrasse on the substrate would be negatively influenced by their respective cleaning rates. The material gleaned from different clients during cleaning interactions, also provides differing nutritional content (Eckes et al. 2015) and thus like other cleaners (*Elacatinus* spp. see Soares et al. 2007; Francini-Filho and Sazima 2008) it was hypothesised that juvenile blue-headed wrasse will clean certain client species more frequently than others to meet their nutritional requirements.

METHODS

Study site and species

Observations on juvenile blue-headed wrasse (*Thalassoma bifasciatum*) and sharknose goby (*Elacatinus evelynae*) cleaning behaviour were collected from Pirates Bay Reef in Charlotteville, Man O' War Bay, Tobago (11° 19' 00" N, 60° 33' 00" W) in January to February 2017 by daily snorkeling between the hours of 08:00 and 17:30. The shallow reef area sampled (30 m x 50 m, reef top depth 0.5 – 2 m) is on the east side of the bay about 100 m offshore. The fringing reef is mainly composed of rocky sandy substrate, the encrusting zooxanthid (*Palythoa caribaeorum*) and living hard coral (*Siderastrea* spp. and *Montastraea* spp., Mallela et al. 2010), providing suitable habitats for sharknose goby cleaning stations (Soares et al. 2008c). These small dedicated cleaning gobies (max 4.5 cm fork length), the predominant Caribbean cleaner fish, are characterised by conspicuous black and blue lateral stripes on the body (Cheney and Côté 2003). They occupy the same reefs as the more facultative juvenile blue-headed wrasse cleaners (max 15 cm fork length). These cleaners are characterised by their yellow body colouration and black spot on the dorsal fin (Feddern 1965).

Cleaner-client interactions

To compare the abundance of juvenile blue-headed wrasse and sharknose gobies on the reef, 30 m x 2 m belt transects (n = 12) were laid along the reef, and the number of each cleaner species were counted along each transect. To quantify wrasse cleaning behaviour, focal individuals (n = 94) were observed for up to 10 minutes or until focal fish were lost. Individual wrasse could not be identified due to their uniform body patterning and free swimming behaviour across the reef, but it is unlikely that the same individual was observed multiple times due to their high abundance on the reef; unpublished fish abundance surveys conducted over the last four years at Pirates Bay Reef have consistently recorded over 150 individuals of free-ranging juvenile blue-headed wrasse per 50 minute survey. To compare cleaning behaviours, at least ten individual sharknose gobies occupying ten marked cleaning stations were observed (n = 10.7 x 10 minute observations per station ± 2.04; mean ± S.E.). The number of gobies occupying these stations ranged from one to seven but only one focal fish was observed at a time, and it is unknown whether the same individual goby was observed multiple times. For both cleaner species, the frequency of cleaning events per unit time was recorded. A cleaning event began when a cleaner and single client physically interacted, ending when either the cleaner or client terminated the interaction (Floeter et al. 2007). In contrast to the sharknose goby cleaners (Côté 2000), juvenile blue-headed wrasse reportedly do not rely solely on gleaned material as a food source (Feddern 1965) and therefore the non-cleaning substrate foraging rate of juvenile wrasse was also recorded during these 10 minute focal observations.

As cleaning was rarely observed during juvenile blue-headed wrasse focal observations, an event sampling method, which is more likely to capture rarer behaviours (Altmann 1974), was also used to quantify the frequency of wrasse cleaning behaviour across the reef. The occurrence of cleaning events by juvenile wrasse was recorded during 10 minute random swims over the reef study area (n = 49). When a cleaning event was observed the species of the client was recorded. At one site on the reef, which was characterised by large flat boulders covered with encrusting *P. caribaeorum* zooxanthid, juvenile blue-headed wrasse cleaning interactions were consistently and frequently observed. To supplement snorkelling observations, two underwater video cameras (QUMOX SJ4000 Action Cams) were used to document wrasse cleaning behaviour at this station over 10 days. Event and focal surveys did not include this cleaning station so that a comparison could be made between juvenile blue-headed wrasse wandering and stationary cleaners (Itzkowitz 1979).

Cleaner-client interactions: video analysis

Following a 30 second period after the observer had placed a camera at the cleaning station, videos (n = 10, ≈ 39 minute per video) were analysed at 1 minute intervals for 10 seconds (n = 359 observations). The number and species of clients posing (stationary postures where an individual presents their body to cleaners, Feder 1966) and the number of cleaning events (visible peck on the client's body) by each juvenile blue-headed wrasse observed in the video were recorded. Due to the static nature of the camera, not all cleaning events could be observed, so it was also recorded when a cleaner associated with a client but subsequently went out of view. The percentage of view blocked by the reef substrate was recorded to account for differences in video position as a result of rugose habitat. Cleaners were also recorded associating with and inspecting clients without cleaning (like in **Chapter 4**).

Data analysis

Data analyses were conducted using the statistical software R, version 3.2.2 (R Core Team 2017). All Generalised Linear Mixed Model (GLMMs) were run using the glmer call in the lme4 package (Bates et al. 2015). All models were refined by stepwise deletion with the removal of non-significant terms. Fit was assessed using residual plots as recommended by Bolker et al. (2009) with all continuous variables standardised to facilitate model convergence.

The total time for each focal observation accounted for the amount of time a cleaner was out of view, and thus varied across observations. A binomial GLMM with a probit link function compared the difference in cleaning frequency, whilst accounting for observation time, between juvenile blue-headed wrasse and sharknose goby cleaners. Due to differences in data collection method for the video observations, this model only considered cleaning frequency across the reef. This data were collected using two methods; focal observations quantified sharknose goby and free swimming juvenile blue-headed wrasse cleaning frequency, whilst event sampling further quantified blue-headed wrasse cleaning. Thus, observation method (focal and event) was included as a random factor to control for these differences in data collection method. This model accounted for repeated observations at the same sharknose goby cleaning station by including station number as a random factor. Cleaner species, time into study period and minutes into day were included in the model, with relevant two-way interactions, as fixed effects. To compare the diversity and evenness of clients, Shannon’s diversity indices were calculated based on average cleaning rates across sharknose goby and juvenile blue-headed wrasse event and focal observations using the 'vegan' package (Oksanen et al. 2013). A Wilcoxon matched pairs test compared cleaner species abundance along each transect.

Client species cleaned were assigned maximum fork lengths using (Humann and Deloach 2014) and recorded as either solitary or gregarious (associate with > 3 individuals) and sedentary or free ranging, using FishBase (Froese and Pauly 2018). To account for overdispersion, a quasibinomial Generalised Linear Model (GLM) with a logit link function determined whether the proportion of cleaning events, observed within each cleaner species, differed towards assigned functional traits of their clients. The two different data collection methods (focal and event) were combined, and the model included the fixed effects: cleaner species and the interactions with client: social behaviour, range and maximum size. Correcting for overdispersion, another quasibinomial GLM with a logit link function determined whether the cleaning rate (accounting for observation length), day into study and minutes into day influenced individual wrasse substrate foraging counts.

For video observations, a Poisson GLMM with a log link function determined whether the number of clients and wrasse cleaners influenced the frequency of observed cleaning interactions. Time into the day (in minutes), time into study and amount of view blocked by substrate were also included in the model as fixed factors. As repeat observations were made within the same video, video number was included as a random effect. A similar model with the same fixed and random terms determined whether juvenile cleaner wrasse and client abundance influenced the number of cleans per client per cleaner. A Gaussian family was specified with an identity link function. Preliminary analyses found the amount of observation view blocked by substrate did not influence observed results and so is not further included here.

RESULTS

Cleaning frequency

Despite being more abundant on the reef than sharknose goby cleaners (median: 6.5 juvenile blue-headed wrasse; 3 sharknose gobies/30 m, Wilcox, $Z = 60$, $N = 12$, $p = 0.018$), significantly more cleaning events were observed by sharknose gobies (0.11 ± 0.01 cleans per minute; mean ± S.E.), compared to juvenile wrasse observed across the reef (0.02 ± 0.004 cleans per minute; GLMM, $\beta = 0.60$, $\chi^2_1 = 20.06$, $p < 0.001$). For both cleaner species, this effect was consistent across the day (GLMM, $\chi^2_1 = 0.377$, $p = 0.539$) and study period (GLMM, $\chi^2_1 = 0.12$, $p = 0.729$). At one location on the reef, however, juvenile blue-headed wrasse cleaning rate (1.57 ± 0.37 cleaning events per minute) was over 14 x greater than all other rates observed for both cleaners across the reef (sharknose goby: 0.11 ± 0.01 cleaning events per minute, juvenile blue-headed wrasse: 0.02 ± 0.004 cleaning events per minute). At this station, wrasse cleaners also inspected clients but did not clean them within the observation period (1.42 ± 0.30 inspections per minute).

Cleaning station

In each 10 second period of video analysed, 4.18 ± 0.18 clients (mean ± S.E., maximum observed = 19 individuals) posed for 1.90 ± 0.06 juvenile blue-headed wrasse cleaners (maximum observed = 5 individuals). Juvenile wrasse only cleaned three species from the same family (*Acanthurus* spp., Figure 3.1) even though 11 client species from seven families posed for the cleaners at this location. When juvenile wrasse cleaners were absent from their cleaning station, posing behaviour ($n = 87$) was still observed by six species (predominantly *Acanthurus* spp.) across all video observations.

Cleaning frequency significantly increased with the number of juvenile blue-headed wrasse cleaners (GLMM, $\beta = 0.49$, $\chi^2_1 = 19.35$, $p < 0.001$) and clients (GLMM, $\beta = 0.29$, $\chi^2_1 = 7.14$, $p = 0.008$) present at the juvenile blue-headed wrasse cleaning station. This effect was not influenced by time of day (GLMM, $\chi^2_1 = 0.81$, $p = 0.369$) or time into the study (GLMM, $\chi^2_1 = 3.22$, $p = 0.727$). However, an increased number of clients (GLMM, $\chi^2_1 = 12.57$, $p < 0.001$) or cleaners (GLMM, $\chi^2_1 = 5.73$, $p = 0.017$) at the wrasse cleaning location (Figure 3.2) resulted in a significant decrease (albeit low R^2 values, Figure 3.2) in cleans per client for each juvenile cleaner present, with the decline more marked when the number of clients increased ($\beta = -0.03$) compared to the number of cleaners ($\beta = -0.02$). There was no interaction effect between the number of juvenile wrasse cleaners and clients at the station (GLMM, $\chi^2_1 = 1.07$, $p = 0.302$). The cleaning rate of each juvenile blue-headed wrasse was not influenced by time of day (GLMM, $\chi^2_1 = 0.11$, $p = 0.746$) but did decrease across the study period (GLMM, $\beta = -0.03$, $\chi^2_1 = 5.31$, $p = 0.021$).

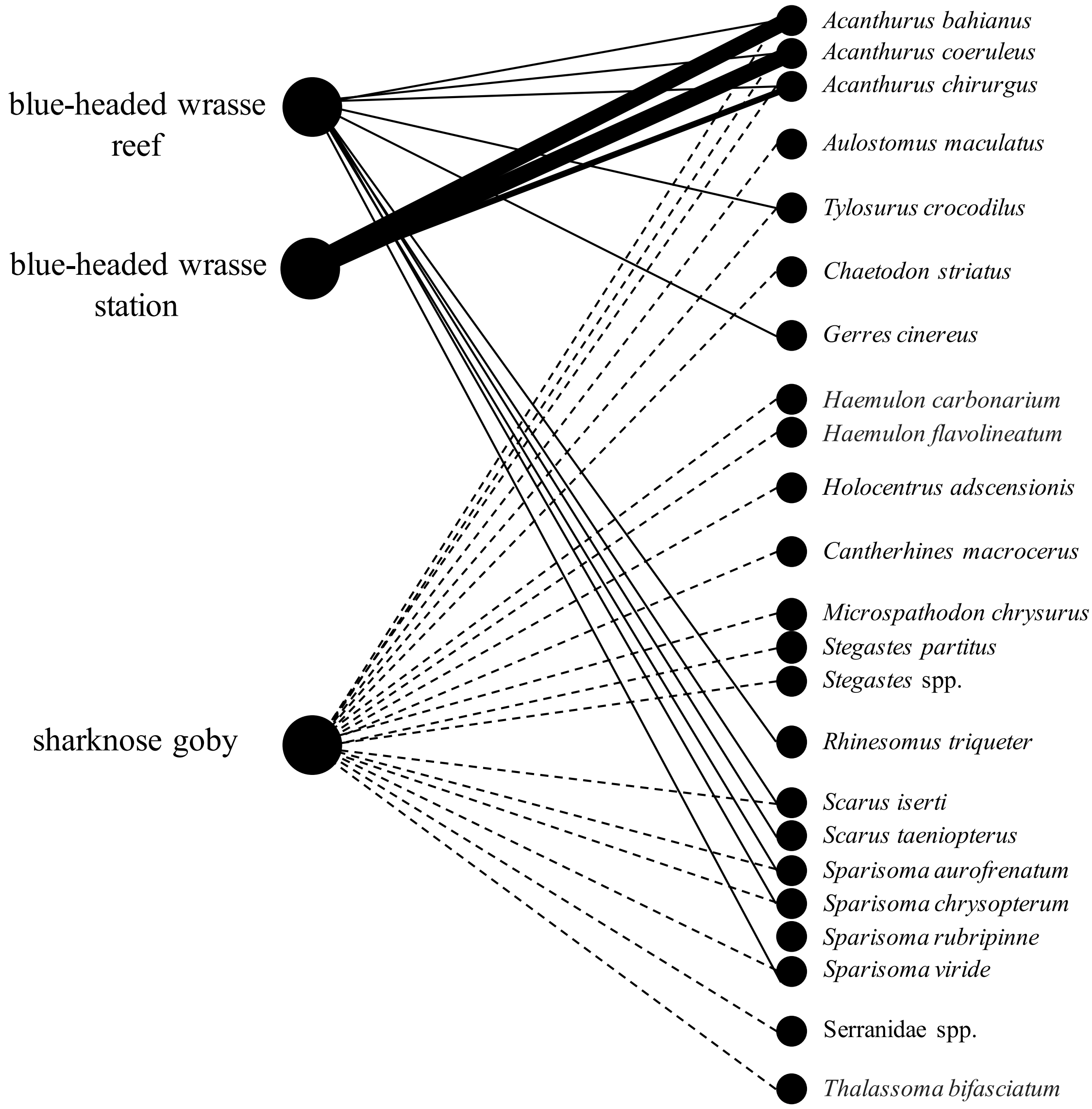


Figure 3.1: Network showing clients cleaned by sharknose goby (*Elacatinus evelynae*) and juvenile blue-headed wrasse (*Thalassoma bifasciatum*) (either swimming free on the reef or at a specific locality). Sharknose gobies were never observed cleaning in open water. Edge thickness represents the cleaning rate per minute for each cleaner and client species. Clients are grouped based on family.

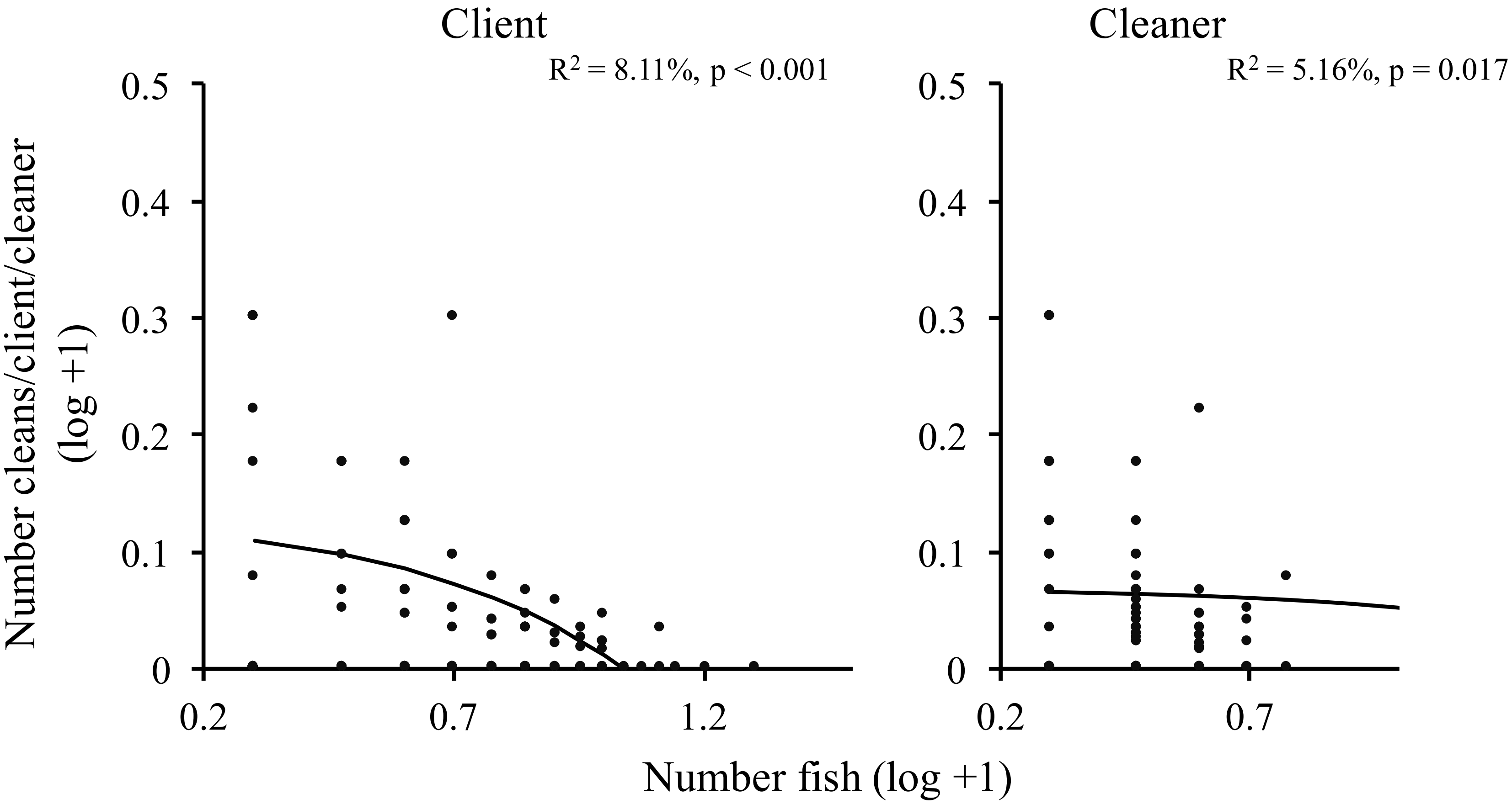


Figure 3.2: Number of cleaning events per client per juvenile blue-headed wrasse (*Thalassoma bifasciatum*) showing a negative relationship with the number of clients and cleaners present.

Substrate foraging

Those juvenile blue-headed wrasse observed cleaning, also foraged on the substrate within the same focal observation period. Cleaning rate was not significantly correlated with the substrate foraging rate of these focal individuals (GLM, $\chi^2_1 = 0.004$, $p = 0.95$). Juvenile wrasse foraged on the substrate at the same rate across the day (GLM, $\chi^2_1 = 1.36$, $p = 0.244$) and study period (GLM, $\chi^2_1 = 3.27$, $p = 0.071$).

Clients

Across the reef, sharknose gobies cleaned a greater diversity of clients at a higher evenness than juvenile blue-headed wrasse (Shannon's diversity = 2.40 cf. 2.03, evenness = 0.69 cf. 0.58, respectively). Sharknose gobies cleaned 19 species from 11 families whilst juvenile wrasse cleaned 11 species from five families, both cleaner species overlapped in the species that they cleaned (Figure 3.1). From snorkelling observations across the reef, juvenile blue-headed wrasse and sharknose gobies cleaned both gregarious and solitary clients (Figure 3.3a) in similar proportions (GLM, $\chi^2_1 = 0.61$, $p = 0.44$). The clients' swimming range, however, did influence the proportion of cleaning events between cleaner species (GLM, $\chi^2_1 = 7.48$, $p = 0.006$), with wrasse only cleaning free-ranging clients, whilst sharknose gobies cleaned both free-ranging and sedentary clients, but a higher proportion of sedentary clients (Figure 3.3b). For both cleaner species, the maximum body size of the client did not influence the proportion of cleaning events received (GLM, $\chi^2_1 = 0.73$, $p = 0.393$, Figure 3.3c). However, juvenile wrasse cleaned clients at the upper end of the sharknose goby client's body size range (Figure 3.3d). From video observations of the cleaning station, juvenile blue-headed wrasse only cleaned three gregarious free-ranging herbivorous species (Figure 3.1) at the lower end of their client size range (30 – 38 cm, Figure 3.3c), despite 11 species posing.

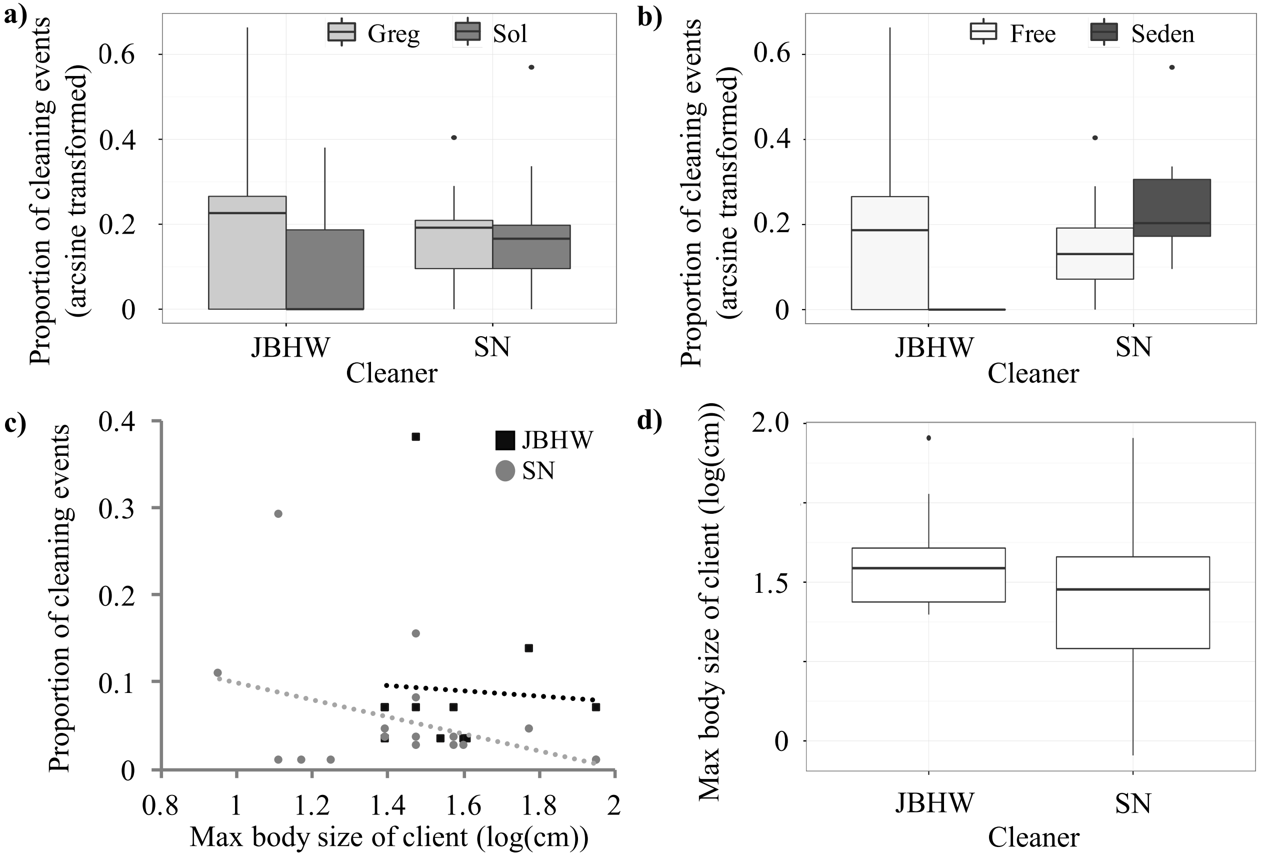


Figure 3.3: Proportion of juvenile blue-headed wrasse (*Thalassoma bifasciatum*) and sharknose goby (*Elacatinus evelynae*) total cleaning events elicited by a) gregarious versus solitary clients with median and inter-quartile ranges (proportion values are arcsine square root transformed), b) free ranging versus sedentary clients with median and inter-quartile ranges (proportion values are arcsine square root transformed) and c) clients of differing max body size on Pirates Bay Reef, Tobago. d) Size range of clients cleaned by both cleaner species, with median and inter-quartile ranges. SN = sharknose goby, JBHW = juvenile blue-headed wrasse.

DISCUSSION

Overall, juvenile blue-headed wrasse cleaned less frequently than sharknose goby cleaners and foraged predominantly on the substrate across the reef. A specific cleaning station facilitated an increased cleaning frequency by these wrasse, where just three larger bodied free ranging client species were cleaned despite posing by a total of 11 species. At this juvenile wrasse station, total cleaning frequency increased with the number of cleaners or clients present, but individual cleaning rate decreased as the number of other fish increased.

This chapter documents a dual cleaning strategy for juvenile blue-headed wrasse (confirming Itzkowitz 1979); cleaning by wandering individuals across the reef and cleaning by individuals or groups at a specific location. For the first time, the frequency of these two different cleaning strategies were quantified. Wrasse cleaning behaviour varied spatially, with a high frequency of cleaning observed at the single cleaning station, whilst cleaning was rarely observed by wandering individuals. In contrast to the numerous sharknose goby cleaning stations on the study reef, the presence of only one juvenile wrasse cleaning station limits knowledge on how widespread the different wrasse cleaning strategies are. However, on a similar sized reef (70 m x 50 m, Booby Reef), again within the Man O' War Bay, one juvenile blue-headed wrasse cleaning station has also been observed. Anecdotal observations at this second cleaning station also showed an increased cleaning frequency by wrasse in comparison to resident sharknose gobies, which in combination with observations by Itzkowitz (1979), suggests that this cleaning strategy adopted by juvenile blue-headed wrasse is more widespread. It is not clear why cleaning was so frequent at the two locations on our study reefs; cleaning stations are usually associated with topological features of the reef (e.g. Potts 1973a), but there was no obvious unique characteristics of observed juvenile blue-headed wrasse stations. Itzkowitz (1979) suggested that juvenile blue-headed wrasse clients do not learn specific locations for cleaning and act opportunistically, but clients were observed posing at the wrasse stations consistently across years in the absence of cleaners, suggesting that the presence of a cleaning station facilitates more frequent access for these cleaners to their clients. Further observations of juvenile blue-headed wrasse cleaning behaviour across multiple reefs would quantify the prevalence of these cleaning stations and help assess their functions. The number of juvenile cleaners at the cleaning station varied (as also shown by Itzkowitz 1979), but given that these cleaners cannot be individually identified, it is unclear whether these were the same or different individuals (Deady et al. 1995), representing individual plasticity or inter-individual differences in cleaning strategies. Thus, a further study where marked individuals are observed, will help to elucidate why and how these part-time cleaning strategies exist.

Previously, it has been suggested that facultative cleaners only use cleaning stations already occupied by dedicated cleaners (Johnson and Ruben 1988; Whiteman and Côté 2002b). In the absence of dedicated cleaners however, facultative cleaners occupy their own stations or share stations with other facultative species (Quimbayo et al. 2012; Walsh et al. 2017). In contrast to Johnson and Ruben (1988) and Walsh et al. (2017), blue-headed wrasse were only observed simultaneously associating with sharknose gobies and clients in 3% of observed cleaning interactions at this juvenile blue-headed wrasse cleaning station, and no such association was observed elsewhere on the reef. Further, no other facultative cleaner species (e.g. *Pomacanthus paru* or *Bodianus rufus*) were observed on the study reef. In aquaculture, where facultative cleaners (e.g. ballan wrasse, *Labrus bergylta*; goldsinny, *Ctenolabrus rupestris* and lumpfish, *Cyclopterus lumpus*) are deployed to biologically control ectoparasites of farmed fish (e.g. sea lice, *Lepeophtheirus salmonis* and *Caligus* spp. infecting farmed Atlantic salmon, *Salmo salar*, see Rae 2002), the simultaneous use of two cleaner species is thought to enhance their cleaning efficiency (Powell et al. 2018). However, from this study it appears that other cleaner species are not required to facilitate facultative cleaning.

This chapter provides the first *in situ* evidence that the local abundance of fish on the reef influences the observed patterns of facultative cleaning for juvenile blue-headed wrasse (as also shown in **Chapter 6** for a dedicated cleaner). At the wrasse cleaning station, the overall cleaning frequency increased with the number of cleaners or clients, whilst the cleaning efficiency of juvenile blue-headed wrasse (cleaning rate per fish) decreased when more fish (both cleaners and clients) were present. Previous *ex situ* studies also show a decreased cleaning efficiency when the number of facultative cleaners increases (Groner et al. 2013; Skiftesvik et al. 2013; Imsland et al. 2014a). A high density of fish may increase the chance of a predatory attack (Wittenberger 1985), or, these facultative cleaners may experience a 'confusion effect', whereby the presence of a large number of individuals may make it difficult for the cleaner to assess the client availability (Krakauer 1995; Ward et al. 2004). In contrast, dedicated cleaners are afforded protection from predators throughout their lifespan (Potts 1973a; Darcy et al. 1974; Losey 1979; Côté 2000) and interact with a large number of clients daily (e.g. *Elacatinus figaro* engage in 110 cleaning interactions per day, Sazima et al. 2000): the cleaning efficiency of dedicated captive barber gobies (*Elacatinus figaro*) increased when the number of clients increased (de Souza et al. 2014), contrasting findings in **Chapter 6**. For cleaners deployed in aquaculture, finding optimal cleaner client ratios (e.g. one cleaner to 20 farmed fish, Skiftesvik et al. 2013) is key to increasing deployed cleaners ectoparasite removal efficiency (Deady et al. 1995; Imsland et al. 2014a; Leclercq et al. 2014; Imsland et al. 2015). However, consideration of the overall numbers of both cleaners and clients in fish farm pens may enhance the efficiency of cleaning rates.

Unlike other facultative wrasse cleaners (e.g. *Thalassoma norohanum* see Francini-Filho and Sazima 2008; Quimbayo et al. 2017, and *Bodianus rufus* see Johnson and Ruben 1988), juvenile blue-headed wrasse clean less frequently than resident dedicated goby cleaners (Johnson and Ruben 1988). When not cleaning, juvenile *Thalassoma* wrasse feed on benthic reef organisms (Feddern 1965; Narvaez et al. 2015) and in this study wrasse predominantly fed on the benthos, and did not alter their substrate foraging rates when also cleaning, suggesting that they are generalist foragers with cleaning only supplementing their diet. For other facultative cleaners (e.g. juvenile *Pomacanthus paru*, Sazima et al. 1999), gleaned material forms a predominant dietary source. Morphologically, juvenile wrasse may not be as efficient at removing parasites compared to more frequent cleaners: *Elacatinus genie* gobies are better at removing monogenean ectoparasites (*Neobenedenia melleni*) from clients than juvenile blue-headed wrasse (Cowell et al. 1993). Additionally, for larger facultative cleaner species, ectoparasite consumption alone does not lead to satiation (Leclercq et al. 2014), and although similar sized dedicated cleaner wrasse (e.g. *Labroides dimidiatus*) do rely on gleaned material (Côté 2000), they can consume up to 1200 gnathiids daily (Grutter 1996a). Thus, nutrition gained from gleaned material is unlikely to replace that gained from substrate foraging for juvenile blue-headed wrasse; hence their minor role as reef cleaners (Johnson and Ruben 1988) and generalist feeding strategy. The lack of relationship between cleaning and substrate foraging rate found here, provides further evidence that these juvenile wrasse clean opportunistically (Itzkowitz 1979; Johnson and Ruben 1988) as an extension of their existing benthic foraging behaviour (Poulin and Grutter 1996). Such opportunistic behaviour is commonly observed by deployed commercial facultative cleaners which "ignore" clients to forage on detritus or commercial feed (Deady et al. 1995; Imsland et al. 2014b; Leclercq et al. 2014).

Rather than relying on cleaning for nutrition, juvenile blue-headed wrasse may instead use cleaning to gain a particular food type. Unlike other more specialised facultative cleaners (e.g. *Pomacanthus paru*, Sazima et al. 1999), and despite being more generalist foragers, these wrasse cleaners were more selective in their cleaning than the resident specialist sharknose gobies, which cleaned a larger client range and all client types and body sizes (as in Darcy et al. 1974; Johnson and Ruben 1988; Francini-Filho and Sazima 2008). Facultative cleaners tend to clean non-threatening, herbivorous and detritivorous clients (e.g. Francini-Filho and Sazima 2008; Quimbayo et al. 2012) and despite 11 species posing for juvenile wrasse, they predominantly cleaned three focal herbivorous species within the surgeonfish family. The predominant cleaning of surgeonfish species; blue tang (*Acanthurus coeruleus*) and ocean surgeonfish (*Acanthurus bahianus*), was also anecdotally observed at another juvenile blue-headed wrasse cleaning station on a different reef in Man O' War Bay. These free-ranging, gregarious clients may host a high prevalence of parasites (Patterson and Ruckstuhl 2013) and/or high quality mucus; surgeonfish mucus has high calorific content (Arnal et al. 2001). Juvenile wrasse were also observed approaching and inspecting these client species but not cleaning (also noted by Feddern 1965; Johnson and Ruben 1988), suggesting that these cleaners are searching for a particular food source on their preferred client species. Different types of gleaned material differ in their nutritional content, and client species vary in the quantities of these materials (Eckes et al. 2015). Unlike the facultative cleaner *Thalassoma lunare*, and more similarly to *Thalassoma klunzingeri* (Barbu et al. 2011), juvenile blue-headed wrasse were unlikely preferentially feeding on client mucus during their cleaning interactions, as clients were rarely observed jolting (client jolts are indicative of cleaners removing scales or mucus, e.g. Barbu et al. 2011). Instead, different client species are infected with specific parasite assemblages (Grutter 1994), which may explain why, body length did not influence juvenile blue-headed wrasse and sharknose goby cleaning rates (confirming Grutter and Poulin 1998b; Arnal et al. 2000). The conspicuous gnathiid and copepod crustaceans are the most cited ectoparasites in cleaning interactions (Cheney and Côté 2003; Côté and Soares 2011), and the role of other ectoparasites, such as monogeneans (Grutter 1994;1995b), remains relatively unexplored. Parasite diversity and abundance varies spatially amongst reefs (Cheney and Côté 2003; Sellers et al. 2015), and if the food source rather than the host species influences the selective cleaning behaviour of this facultative cleaner, this may explain why different studies have recorded variable frequencies of their cleaning (Feddern 1965; Darcy et al. 1974; Walsh et al. 2017). Molecular analyses of stomach content would provide useful nutritional information on juvenile blue-headed wrasse diet to further quantify the importance of cleaning to this facultative species.

This chapter suggests that cleaning is not key for juvenile blue-headed wrasse nutrition, which explains why they only adopt a minor cleaning role on the reef. Although these cleaners are opportunistic adopting a more generalist foraging strategy, they are also selective in their client choice, suggesting that the availability of certain supplementary food types, may be driving the frequency of their cleaning behaviour. A specific cleaning station facilitated juvenile blue-headed wrasse cleaning frequency, providing a stable location for visiting clients. However, the presence of more clients or cleaners inhibited wrasse cleaning rates restricting the facultative nature of their cleaning behaviour. Cleaning behaviour observed for this wrasse species, appears to parallel cleaning observed in aquaculture where maintaining and enhancing the feeding efficiency of deployed cleaner fish is a major challenge (Imsland et al. 2016). Like the wandering juvenile blue-headed wrasse, deployed cleaners are facultative, rarely adopting cleaning behaviours in their natural environments (Potts 1973b), suggesting that perhaps the cleaning efficiency of existing deployed cleaner species cannot be substantially increased, and alternative species should be sought. Future work should determine the food types (e.g. parasites) driving the selective nature of juvenile wrasse cleaning behaviour and identify whether certain habitat features of cleaning stations determine spatial variation in cleaning.

Chapter 4

To Clean or Not to Clean: Cleaning Mutualism Breakdown in a Stressed Environment

A version of this chapter is in preparation for journal submission.

ABSTRACT

Mutualistic interactions involve two species beneficially cooperating, but it is not clear how and why these interactions are maintained. Partners could gain greater rewards if they overexploit or abandon the mutualism, but this is not commonly observed. The dynamics and pervasiveness of mutualisms are regulated by context-dependency, and these interactions, which can provide key ecosystem services (such as pollination and health regulation), are not immune to breakdowns. This chapter hence investigated cleaning patterns of a model cleaner fish species, the bluestreak wrasse (*Labroides dimidiatus*), in an isolated tidal lagoon in the Great Barrier Reef, to investigate the stability of a mutualism within a stressed environment. Tidal environments are naturally stressed systems which may mimic changing environmental conditions. Bluestreak wrasse are renowned dedicated cleaners, relying solely on client gleaned material for food. Instead this study found that under stressed natural conditions, both adults and juveniles were part-time facultative cleaners, also pecking on the coral *Isopora palifera*. Despite coral feeding the most, adult cleaners did not abandon the mutualism altogether, but wandered across the reef in search of clients, rather than waiting at fixed sites, often referred to as 'cleaning stations'. Juveniles appeared to exploit the system more, by biting their clients more frequently than adults at cleaning stations. Within this naturally stressed system, the frequency and dynamics of this cleaning mutualism may thus be breaking down, with mutualistic cleaning not representing a stable food acquisition behaviour. Given this response for a dedicated cleaner there is now concern about what will happen to the pervasiveness of, the equally important and more ubiquitous, facultative mutualists under changing environmental conditions.

INTRODUCTION

The beneficial mutualistic interactions between species are governed by conflicts of interest (Sachs and Simms 2006). Such service-resource interactions involve one member gaining food resources (e.g. nectar or ectoparasites) and the other receiving a beneficial act (pollination, Landry 2012, or parasite removal, Grutter 1996a). In these interactions, however, partners will benefit more if they exploit or abandon the other, so it is difficult to understand how these mutualisms are so ubiquitously maintained. Mutualisms are not immune to breakdowns, which can be defined as the loss of cooperative phenotypes in a mutualists' lineage over time (Sachs and Simms 2006). Given the key ecosystem services these interactions can provide (e.g. pollination, Landry 2012, and health enhancing parasite control, Clague et al. 2011b), it is crucial to understand what supports mutualism stability. It is well established that mutualisms are context-dependent, with their dynamics being influenced by abiotic and biotic conditions (Chamberlain et al. 2014). Differing contexts will hence influence the magnitude of benefits each interacting partner gains, facilitating how they behave (Frederickson 2017). Given that climate change and biodiversity loss are two major global environmental challenges (Skogen et al. 2018), it is unclear how mutualisms will function under stressed environmental contexts.

Behavioural changes play a leading, first response, role for species attempting to adjust to more challenging environmental conditions, facilitating their survival (Wong and Candolin 2015). One way animals can rapidly adjust to changed/fluctuating environments is through phenotypic plasticity, in other words, the tendency of a genotype to produce different phenotypes under asymmetric environments (Thibert-Plante and Hendry 2011). Plasticity allows animals to adjust their traits to their immediate environment, increasing their fitness (van Buskirk 2012). Many species can adapt their foraging behaviours, for example, in response to rapid environmental changes (Gilmour et al. 2018). For dedicated (formerly obligate) full-time service providers, they rely entirely on mutualistic interactions for food (Vaughan et al. 2017). This behaviour is considered a specialist foraging strategy (**Chapter 3**): specialist species prefer specific food types or have a narrower dietary range, whilst generalists have a diverse diet or consume a broad dietary niche (Bridcut and Giller 1995; Amundsen et al. 1996). Specialist foraging strategies are, however, only adaptive when resource competition is low (Stephens and Krebs 1986), i.e. there is a plentiful and stable supply of food (West-Eberhard 1989), so are not expected to persist in unstable environments. It is unknown what will happen to these mutualists in changing environments, will they shift their behaviour to become more parasitic gaining better food rewards? Will they abandon the mutualism? Or given their long-established, narrow dietary range, will they become extinct (Sachs and Simms 2006)? There are current examples of all three of these responses (shift to parasitism, e.g. nectar robbing, Irwin et al. 2010, abandonment, Pellmyr et al. 1996, and extinction, Ollerton et al. 2014). So, what will happen to mutualists under the rapidly changing environmental conditions our ecosystems are now facing?

In addition to behaviour and diet changes, animals can adapt their habitat use as a rapid response to a changing environment (van Buskirk 2012). Habitat use can be an important facilitator of mutualistic interactions. Cleaner fish for example, which remove ectoparasites and other material from the body of another fish (termed a client, Feder 1966), can associate with topological features of the environment that form cleaning stations (Potts 1973a). Cleaners wait at these stations for clients to visit and cleaners that do not occupy fixed cleaning stations show reduced cleaning behaviours, and hence feeding opportunities (Mills and Côté 2010; Oates et al. 2010).

The cleaner-client interaction is a model system for investigating the stability of mutualisms in changing environments. Cleaning is ubiquitously observed on coral reefs (White et al. 2007), environments that are particularly vulnerable to global change. The reef building Scleractinia stony corals are fragile to environmental anomalies (Hoegh-Guldberg et al. 2007) and their decline, with other reef builders, results in large changes in the associated reef fish community (Munday 2004). Many studies focus on how coral symbioses will respond to climate change by investigating their communities in present day stressed reefs, where they are exposed to highly variable tidal environments (Oliver and Palumbi 2011). Tidal lagoons, found within coral reef atolls, thus provide a "Sentinel System" to investigate how cleaning symbioses persist in stressed environments. Like coastal lagoons, atolls can be isolated from the surrounding ocean for periods of the day, leading to elevated temperature, low pH and low dissolved oxygen, periodically fluctuating with tidal and diurnal cycles (Silverman et al. 2012). These naturally stressed systems may mimic future environmental conditions (Camp et al. 2017), providing a unique environment to examine phenotypic changes and adaptive shifts in mutualistic cleaning.

The Great Barrier Reef is the world's largest and most intensively managed coral reef ecosystem. It is currently experiencing high levels of stress and change from multiple sources including cyclones, crown of thorn starfish (*Acanthaster planci*), pollution and warming events (De'ath et al. 2012), and recently, it has experienced its worst coral bleaching event in history with 90% of the reefs being affected (Hughes et al. 2017). This chapter thus documents the cleaning behaviours and habitat use of the model Indo-Pacific cleaner species, the bluestreak wrasse (*Labroides dimidiatus*) within a tidally controlled lagoon on the Great Barrier Reef, to investigate whether cleaning will function adaptively under stressed environmental conditions. These dedicated cleaners are thought to rely exclusively on client gleaned material as a food source throughout their life (Vaughan et al. 2017), so the behaviours of adults and juvenile wrasse were compared, to determine whether adults show plasticity in their mutualistic behaviour. Given the links between cleaning and habitat, it was first determined whether adults and juveniles differed in their habitat use on the reef. Bluestreak wrasse usually only wait at cleaning stations for clients to visit them, whilst other wrasse cleaner species 'wander' across the reef in search of clients (**Chapter 3**; Oates et al. 2010). In a more limited environment, it was expected that, as also observed by Wilson et al. (2014), bluestreak wrasse would adopt this wandering behaviour, with adults wandering for longer than juveniles. Secondly, a potential function of this wandering behaviour was investigated; to seek out food. Individual adult cleaner wrasse consume high numbers of parasites daily (up to 1200, Grutter 1996a), so need to interact with clients frequently to fulfil their nutritional requirements (each wrasse can interact with clients up to ~2300 times per day, Grutter 1996a). It was therefore hypothesised that wandering cleaners would clean clients for longer and interact with clients more frequently, maintaining a stronger mutualistic interaction. Conversely, cleaners could gain more nutrition from cheating clients and exploiting the mutualism (Eckes et al. 2015). Cheating is a parasitic behaviour that involves a cleaner biting and removing skin from the client whilst cleaning (Cheney and Côté 2005). Under 'normal' conditions, wrasse balance their mutualistic versus parasitic cleaning behaviours to ensure the mutualism is maintained (Bshary 2002a; Binning et al. 2017b), but in this more stressed environment it was expected that cleaning would become more parasitic, breaking down the mutualism (i.e. high biting frequency with low cleaning frequency and duration). Searching for food, rather than waiting for it to arrive (i.e. at stations) is more energetically costly and risky, so cleaners were expected to cheat more when wandering. Given that clients remember the locations of more favourable cleaners and punish cheaters (Bshary and Schäffer 2002), cheating whilst reef wandering would still maintain the occurrence of mutualistic interactions at cleaning stations. Overall, cleaning was expected to become more parasitic in this stressed environment. Bluestreak wrasse have not been documented to abandon cleaning all together, even under home aquarium conditions (instead they do not acclimatise well and have high mortality, Wabnitz 2003), so a mutualism abandonment was not hypothesised to occur.

METHODS

Naturally stressed reef environment

Bluestreak cleaner wrasse (*Labroides dimidiatus*) habitat use and cleaning behaviour were observed in March 2018 on One Tree Reef (23°30′ S, 152°06′ E) situated in the Capricornia Cays National Park in the Southern Great Barrier Reef (Figure 4.1a). One Tree Reef is characterised by three shallow lagoons with high and unbroken reef crests, such that the first lagoon (Figure 4.1a) is isolated from the surrounding ocean for half of each tidal cycle. Due to this isolation, the tide within this first lagoon never drops below 1.52 m (Figure 4.1b, Ludington 1979). The study was conducted in this first lagoon (depth 0.5 – 2.5 m) on cleaners occupying shallow coral bommies on patch reef close to the coral cay, One Tree Island (Figure 4.1a), base of the University of Sydney's Research Station. Low tide residual drainage from the lagoon meant that reef peaks could, at times, be above the water surface (Kinsey 1978; Ludington 1979). Behavioural observations (n = 179) conducted by snorkelling in daylight, between 6am and 6pm, mostly occurred when the tide was flowing (Figure 4.1b). The reef area was predominantly characterised by sand (mean ± S.E. proportion of 12 x 30 m benthic transects: 40.2% ± 5.42) followed by hard corals (32.4% ± 4.36). As a result of tidal changes, the salinity and temperature of the study environment would have also fluctuated: in One Tree Reef's first lagoon, close to the study site, temperature and salinity can decrease when the water level increases within the lagoon (Silverman et al. 2012).

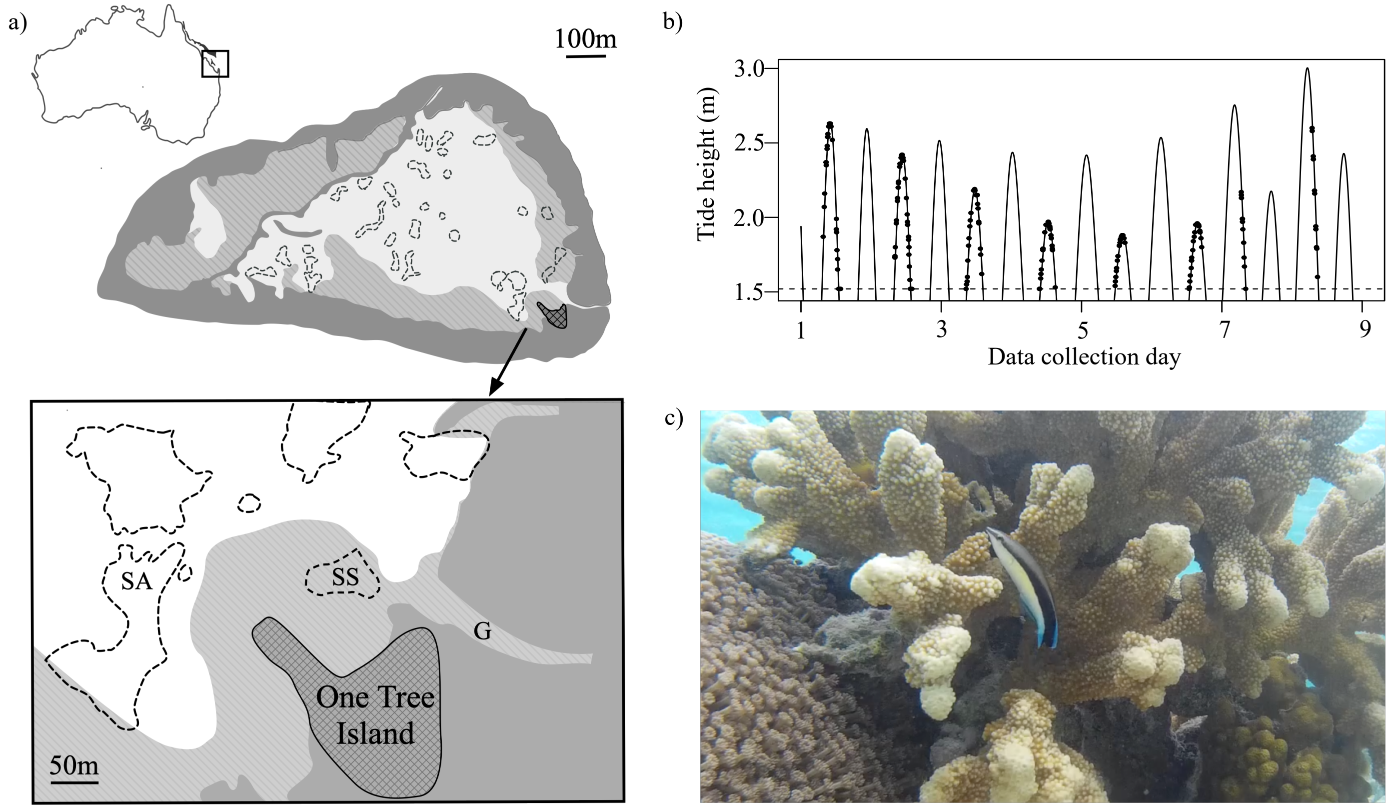


Figure 4.1: Bluestreak wrasse (*Labroides dimidiatus*) study was conducted within the first lagoon of One Tree Reef, Great Barrier Reef (a). The work took place at the Study Site (SS), which was close to the gutter (G). Shark alley (SA) has been added for reference. Light grey area represents sandy substrate, whilst darker grey represents atoll edges and barriers to the surrounding ocean. (b) Diurnal tidal range over which the study took place. Points indicate observation timepoints. Dotted line represents minimum tide height of 1.52 m. (c) Bluestreak wrasse associating with the coral *Isopora palifera*.

Cleaner habitat use in a naturally stressed environment

Adult and juvenile bluestreak cleaner wrasse are morphologically distinct: adults are identified by blue and yellow colouration separated by a longitudinal black line (fork length: 6 – 10 cm), whilst juveniles are characterised by a black body and blue dorsal line (fork length: 2 – 3 cm, Potts 1973a). Adult and juvenile cleaners maintain separate cleaning stations which were identified by repeatedly observing the locality of individuals on the reef in the first two days before data collection. Cleaning station locations were numbered and marked to allow for repeated observations (adults n = 6 marked stations, juveniles n = 13). Within the study site (SS, Figure 4.1a), all adult stations were marked, but it was not possible to mark all juvenile stations due to the high number of individuals, and the frequent appearance of new stations. Adults can share their station with another adult, whilst juveniles are often observed sharing their stations with 'wigglers' – the smallest recognised size category of bluestreak wrasse (mean fork length: 10 mm, Potts 1973a). The behaviour and presence of wigglers was not recorded here due to their size and tendency to seek cover. The repeated measures of cleaner behaviour from the same station likely represent the same individual for juveniles and one of two individuals for adults (it was not possible to naturally identify individuals).

For each cleaning station, habitat characteristics were quantified using standardised 'Habitat Assessment Scores' (following Gratwicke and Speight 2005) which categorically measures rugosity, variety of growth forms, refuse size categories, percentage of live cover and the percentage of hard substratum: higher scores represent more complex habitats. The depth, height and width of each station were also measured (to the nearest cm) to calculate ellipsoid station volumes (after Adam 2011). When a cleaner occupied a station, which consisted of two or more closely isolated reef structures, ellipsoid volumes were calculated separately for each and summed.

Cleaners did not spend all their time at their cleaning stations, and like Wilson et al. (2014) who also observed bluestreak wrasse on One Tree Reef, some fish travelled (hereafter termed 'wandering', see **Chapter 3**) considerable distances from their cleaning stations (> 20 m). Thus within 20 minute observations, the time cleaners spent at their station versus time spent wandering was also recorded. Cleaners were also observed visiting other marked stations but given that all juvenile stations were not marked on the reef, and this behaviour was uncommon (observed in 16.8% of observations, accounting for 3.8% of total observation time), this behaviour was combined with wandering.

Cleaner behaviour in a naturally stressed environment

Both adult and juveniles were observed for up to 20 minutes or until the fish was lost (minimum observation time included = 105 seconds, adults = 64 observations totalling 17 hours, 53 minutes and 18 seconds, juveniles = 115 observations totalling 34 hours, 56 minutes and 32 seconds). Fish observations began at marked cleaning stations (n = 8.52 ± 0.71, mean ± S.E. observations per cleaning station). All behaviours were recorded by observers (n = 3), and observers maintained a distance of 1.5 m from the cleaner.

During each observation, any cleaning interactions were recorded: the number of cleaning events, the time a cleaner spent associating with each client during cleaning, and the occurrence of cheating (biting identified through client jolting, Bshary and Grutter 2002a). Cleaners could also interact with clients without cleaning, either through inspections or by chasing, so the combined frequency of these non-cleaning client interactions were also recorded (like in **Chapter 3**). Since, bluestreak wrasse cleaning behaviour can be affected by the presence of a conspecific (Gingins and Bshary 2014), the time focal cleaners spent with conspecifics was all recorded. All behaviours were separated as to whether they occurred at the cleaning station or when wandering.

During preliminary observations, both age groups of cleaners were observed pecking on *Isopora palifera* (Figure 4.1c, see https://youtu.be/MJ5EQb2bJIE, morphological identification, formerly *Acropora palifera*), a common shallow water reef building branching coral that dominates low energy lagoon environments, including One Tree (Ayre et al. 1991; Benzie et al. 1995). *Isopora* coral is at high risk from bleaching induced extinction (Muir et al. 2017). Cleaners interactions with *I. palifera* were thus also recorded: pecking on *I. palifera* and how much time it spent associating with this coral (swimming within 5 cm, Figure 4.1c). No other significant coral pecking on any other coral species was observed. Again, this behaviour was separated by whether the cleaner was at their station or wandering across the reef. When quantifying cleaning station characteristics (*see above*) one observer also estimated (to the nearest 5%) the percentage cover of *I. palifera* within each station. Any instances of intra-specific cleaning between individuals and any cleaner substrate flashing was also recorded. Flashing involves a fish swimming towards an inanimate object with a curved body position and contacting the object with the convex portion of their body (see https://youtu.be/SNelefHvEM0). Like cleaning, flashing links to hygiene regulation and in particular, the dislodging of ectoparasites (Wyman and Walterswyman 1985).

Data analysis

All analyses were carried out in R, version 3.4.3 (R Core Team 2017) with the additional use of ‘coin’ (Hothorn et al. 2006), ‘gamlss’ (Rigby and Stasinopoulos 2005), ‘lme4’ (Bates et al. 2015), ‘multcomp’ (Hothorn et al. 2008) and ‘TideHarmonics’ (Stephenson 2016). Model assumptions and fits were assessed using residual plots and all continuous predictors were standardised to facilitate model convergence. Data were collected by three observers so ‘ObserverID’ was included as a fixed effect in all models. Where there was significant variation between observers, which could have simply represented the natural variation in cleaning behaviour (since all observed all cleaning stations), results are reported with and without inclusion of ‘ObserverID’. This demonstrated whether results were still consistent even when excluding this source of variation. Time of day and tide height were included as an interaction term in all models. Mean tide heights were calculated using ‘TideHarmonics’, for each observation based on hourly tide predictions for One Tree Island (obtained from: Australia’s Bureau of Meteorology) and the start and end times of each observation. Any tides heights that were predicted to be less than 1.52 m by ‘TideHarmonics’ were recoded to 1.52 m as this represents the lowest tide for the lagoon (Ludington 1979). Tide water flow direction was also assigned (incoming versus outgoing), which was specified as a fixed effect in all models. To account for potential effects of the social environment on observed behaviours, the proportion of time a cleaner spent alone was also included as a fixed effect in all models. Tukey’s tests were used for *post-hoc* analyses.

Analyses used a combination of General Linear Models (GLM), binomial Generalised Linear Mixed Models (GLMM) with a logit or probit link, and beta binomial generalised additive models for location, scale and shape (GAMLSS). Beta binomial GAMLSS models replaced overdispersed binomial GLMMs. GLMs and GLMMs were refined by removing the least significant term in each step, leaving only the best model with significant predictors. In all mixed models ‘StationID’ was specified as a random term to account for repeated observations at stations. GAMLSS model selection was based on Akaike Information Criterion (AIC) using the stepGAIC call. For all models, the significance of fixed effects was assessed using likelihood ratio tests. Due to fish being lost temporarily (cleaner out of view) and/or permanently, before observations ended, observation lengths varied (mean ± S.E. observation length = 1062.51 seconds ± 22.82). Variable observation lengths were accounted for by including frequencies and durations as proportions or including observation length as a fixed effect when a binary outcome was specified as a response variable.

Cleaning station characteristics (n = 19) were quantified using: total HAS scores, ellipsoid volumes and *Isopora palifera* percentage cover. Three separate GLMs determined whether cleaning stations differed between adults and juveniles. For these gaussian (with identity link) GLMs, *I. palifera* cover was arcsine transformed whilst volumes were square-root transformed. Linear discriminant analysis (LDA) identified which was the most important characteristic distinguishing adult versus juvenile stations, and LD1 scores for each station determined whether adults of juveniles were more variable in their station characteristics (Euclidian nearest neighbour distances between LD1 scores modelled using gaussian GLM with identity family). The proportion of time a cleaner spent wandering was calculated for each observation and specified as a GAMLSS response variable (with a beta inflated distribution, BEINF). To improve model fit, 'nu', 'tau' and 'sigma' parameters were specified using stepGAIC (Rigby and Stasinopoulos 2005). To determine whether adults and juveniles differed in their time spent wandering, age was specified as a fixed effect.

To test whether cleaning behaviours differed between wandering versus occupying a station, cleaning frequencies, durations, non-cleaning interaction frequencies and bite tendency (0 = not observed, 1 = observed) were separated into where it occurred on the reef for each observation. This meant that 'ObservationID' was now included along with 'StationID' as random terms. Four separate models (cleaning frequency, cleaning duration and non-cleaning interaction frequency as response variables in beta binomial GAMLSS, and bite tendency as a binomial GLMM response variable) determined whether cleaning behaviours differed between adults and juveniles. Age and location were included as an interaction term in all four models. In the model investigating differences in bite tendency respective cleaning frequencies and durations were also included as fixed effects to determine whether biting ('cheating') likelihood increases with reduced cleaning.

As both adult and juvenile bluestreak wrasse were observed associating with, and pecking at, *I. palifera,* it was determined whether adults and juveniles differed in their interactions with *I. palifera* and whether these interactions related to differences in cleaning behaviours. For each observation, the time the cleaner spent associating with *I. palifera* was calculated (modelled using a beta binomial GAMLSS) and observations were assigned as to whether individuals were observed pecking at *I. palifera* or not (modelled using a binary binomial GLMM). The proportion of observation the cleaner spent wandering, observation cleaning frequency and observation total cleaning duration were specified as fixed effects. When analysing *I. palifera* peck tendency bite tendency was also included (binary coded, 1 = biting observed).

Finally, it was determined whether observed flashing behaviour and intra-specific cleaning was more likely with increased cleaning frequencies, durations and time spent associating with *I. palifera*. Due to the rare occurrence of these behaviours, observations were assigned as to whether flashing was observed or not, and whether intra-specific cleaning was observed or not, and test statistics were generated using permutation tests. To account for repeated measures, data were stratified by 'StationID'. To determine whether flashing or intra-specific cleaning was more likely to occur when *I. palifera* pecking occurred, a Pearson's Chi-squared test with Yates continuity correction was used.

RESULTS

Cleaner habitat use in a naturally stressed environment

Compared to juveniles, adult bluestreak wrasse (*Labroides dimidiatus*) occupied larger cleaning stations that contained a greater percentage covering of *Isopora palifera* (Figure 4.2, station volume GLM, $F_1 = 7.62$, $p = 0.013$, $_{\text{model}}R^2 = 30.9\%$, *I. palifera* percentage GLM, $F_1 = 5.05$, $p = 0.038$, $_{\text{model}}R^2 = 22.9\%$). The habitat complexity (HAS) of the stations, however, did not differ between adult and juvenile bluestreak wrasse (GLM, $p > 0.05$).

Station volume	$3.13m^3 \pm 1.16$	$0.81m^3 \pm 0.18$
Isopora palifera	35.9% ± 14.7	13.3% ± 3.7

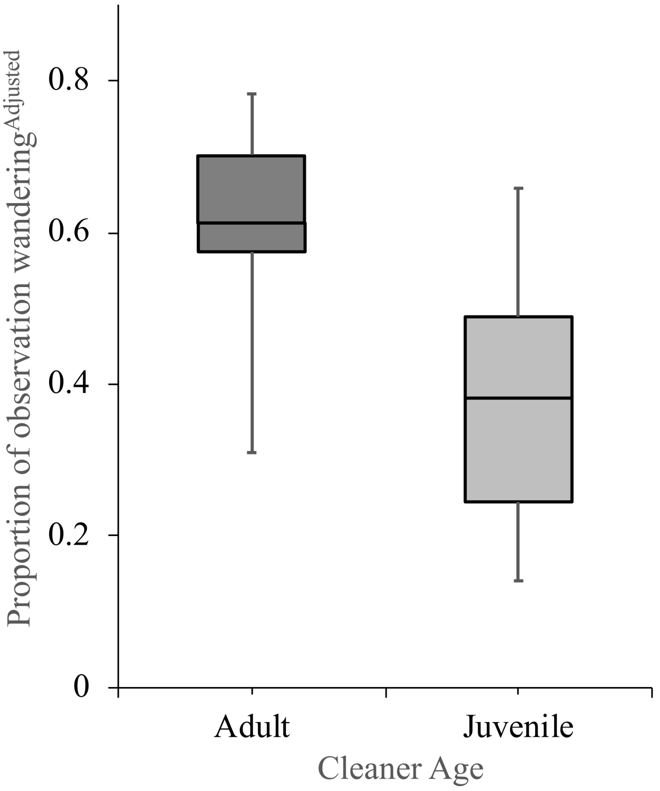


Figure 4.2: Adult bluestreak wrasse (*Labroides dimidiatus*) spent more time wandering from their cleaning stations than juvenile bluestreak wrasse on One Tree Reef, Australia. Boxplot presents median and inter-quartile ranges of response values from GAMLSS model, along with maximum and minimum proportions. Station volume represents the mean ± S.E. station ellipsoid volume of adults versus juvenile stations, whilst *Isopora palifera* shows mean ± S.E. estimated percentage *I. palifera* coverage on stations.

Station size was the biggest discriminator between juvenile and adult cleaning stations (linear discrimination coefficients: volume = 1.31, *I. palifera* percentage cover = 1.14, HAS score = -0.15), and adults were more variable in the types of habitat they occupied as stations (GLM, $F_2 = 59.27$, $p < 0.001$, ${}_{\text{model}}R^2 = 41.4\%$, mean ± S.E. distance between individuals in linear discriminant one values, adults = 1.23 ± 0.19, juveniles = 0.42 ± 0.03).

In addition to being more variable in their station characteristics, adults wandered more frequently across the reef: they spent over half of their time wandering away from their stations (Figure 4.2, GAMLSS, $\chi^2_1 = 6.64$, $p < 0.001$, ${}_{\text{model}}R^2 = 64.9\%$).

Cleaner behaviour in a naturally stressed environment

Across 52 hours 49 minutes and 50 seconds, 2803 cleaning interactions were observed. Juveniles cleaned more frequently and spent more time cleaning than adults (Figure 4.3). Within age groups, juveniles spent more time cleaning when at their stations, whilst wandering adults spent more time cleaning (Figure 4.3, frequency GAMLSS, $\chi^2_1 = 4.03$, $p = 0.045$, ${}_{\text{model}}R^2 = 30.5\%$, $p = 0.043$ without including significant observer effect, duration GAMLSS, $\chi^2_1 = 23.07$, $p < 0.001$, ${}_{\text{model}}R^2 = 37.0\%$). Wrasse also tended to spend more time cleaning at higher tides (Figure 4.3, GAMLSS, $\beta = 0.11$, $\chi^2_1 = 3.76$, $p = 0.052$).

In addition to cleaning, 2717 interactions which involved a cleaner inspecting or chasing a client were observed. These inspection/food searching interactions did not result in cleaning, and were more frequently observed when the cleaner was wandering compared to when it was occupying its station (GAMLSS, $\chi^2_1 = 8.65$, $p = 0.003$, ${}_{\text{model}}R^2 = 7.3\%$, $p = 0.003$ without including significant observer effect, mean inspection frequency ± S.E (weighted by observation length in seconds), station = 0.016 ± 0.002, wandering = 0.019 ± 0.002).

Cheating is a parasitic cleaning behaviour which involves a cleaner biting a client. Here, cleaners were more likely to bite clients when they cleaned more frequently (GLMM, $\beta = 1.80$, $\chi^2_1 = 54.66$, $p < 0.001$, ${}_{\text{model}}R^2 = 67.9\%$, $p < 0.001$ without including significant observer effect). Adults and juveniles also differed in their probability of cheating; juveniles were more likely to cheat clients when at their stations compared to adults occupying stations (Figure 4.3, GLMM, $\chi^2_1 = 4.32$, $p =$

0.038, $p = 0.050$ without including significant observer effect). Cheating probability did not depend on tide in the earlier hours of the day, whilst later in the day, wrasse were more likely to bite at lower tides (Figure 4.3, GLMM, $\chi^2_1 = 4.95$, $p = 0.026$, $p = 0.079$ without including significant observer effect).

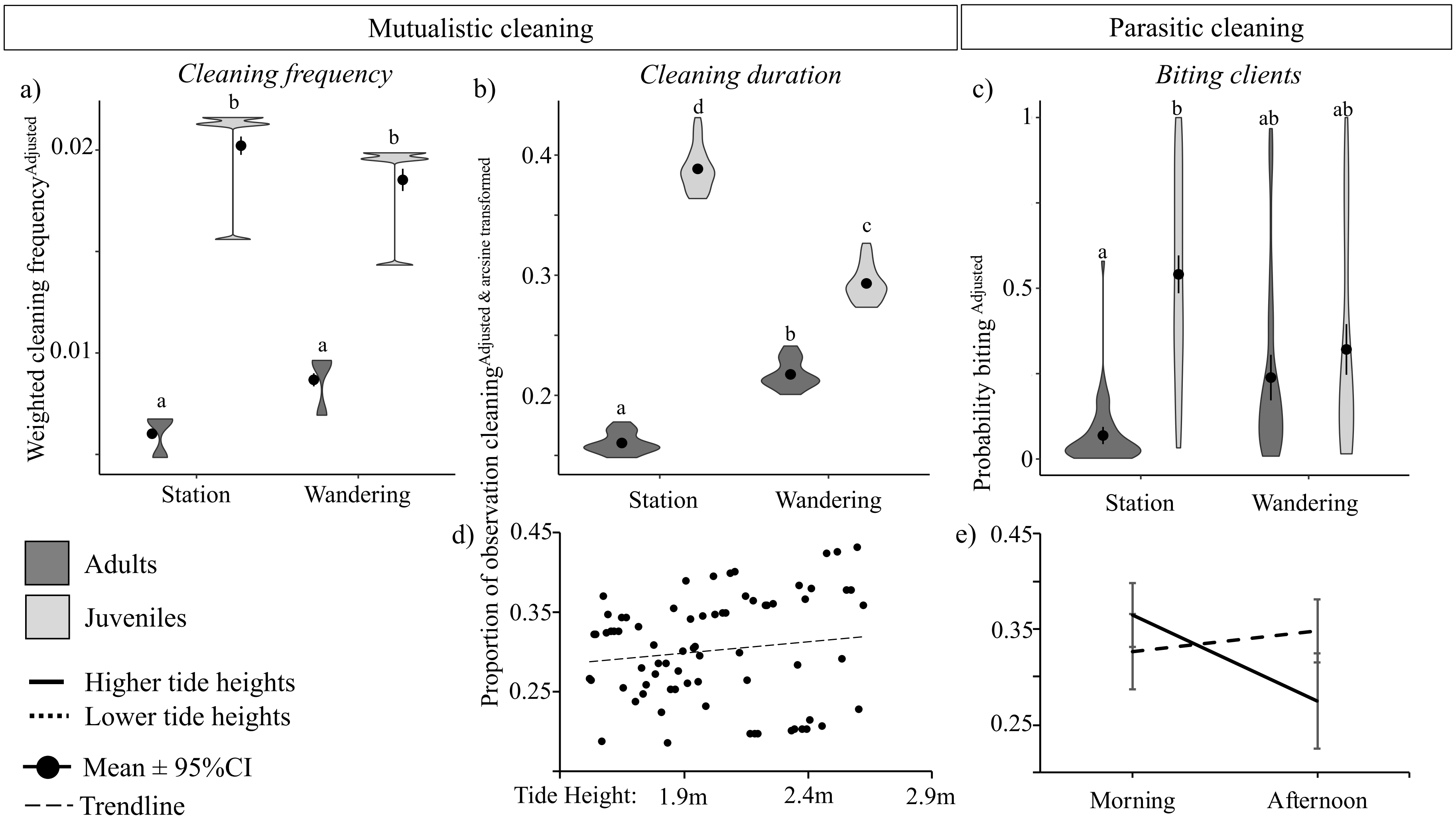

Mutualistic cleaning
Parasitic cleaning
a)
Cleaning frequency
Weighted cleaning frequency Adjusted
0.02
0.01
b
b
a
a
Station
Wandering
b)
Cleaning duration
Proportion of observation cleaning Adjusted & arcsine transformed
0.4
0.3
0.2
d
c
b
a
Station
Wandering
c)
Biting clients
Probability biting Adjusted
1
0.5
0
a
b
ab
ab
Station
Wandering
Adults
Juveniles
Higher tide heights
Lower tide heights
Mean ± 95%CI
Trendline
d)
0.45
0.35
0.25
Tide Height:
1.9m
2.4m
2.9m
e)
0.45
0.35
0.25
Morning
Afternoon

Figure 4.3: Mutualistic and parasitic cleaning behaviours of adult versus juvenile bluestreak wrasse (*Labroides dimidiatus*) wandering across the reef or occupying cleaning stations in a naturally stressed tidal lagoon. The shape of the violin plots represents the observed range of a) cleaning frequencies, b) cleaning durations and c) biting 'cheating' likelihood (adjusted response values from GAMLSS models and a GLMM), whilst shape thickness shows how frequently these data values occurred. Point and lines show mean ± 95% CI. Cleaning duration data is arcsine transformed for figure clarity. Letters represent significance groupings based on Tukey's test and $p < 0.05$. d) Shows the trend between tide height and time spent cleaning ($p = 0.050$), whilst e) represents the significant interaction between mean tide height and time of day and shows their effect on wrasse biting probability (error bars show standard error around the mean value). For analyses, tide height and time of day were considered as continuous predictors but are presented categorically here to show effect.

A reduced reliance on cleaning behaviour?

In addition to cleaning, cleaners were observed interacting with *I. palifera* coral: cleaners associated with, inspected, and/or pecked at, the coral branches (see https://youtu.be/MJ5EQb2bJIE). Adults spent more time associating with *I. palifera* than juveniles (Figure 4.4, GAMLSS, $\chi^2_1 = 4.78$, $p = 0.029$, ${}_{\text{model}}R^2 = 34.7\%$, $p = 0.005$ without including significant observer effect) and this coral association was unaffected by cleaning behaviour (frequency and duration) and reef location (wandering vs station, GAMLSS, all $p > 0.05$). Cleaners that spent more time alone spent more time associating with *I. palifera* (GAMLSS, $\beta = 0.33$, $\chi^2_1 = 6.29$, $p = 0.012$, $p = 0.042$ without including significant observer effect).

I. palifera pecking was observed in 43% of observations and adults were the most likely to coral peck (Figure 4.4, GLMM, $\chi^2_1 = 13.27$, $p < 0.001$, ${}_{\text{model}}R^2 = 71.8\%$). Cleaners which cleaned less frequently were also more likely to coral peck (Figure 4.4, GLMM, $\beta = -1.10$, $\chi^2_1 = 6.40$, $p = 0.011$). *I. palifera* pecking was more likely to be observed in the morning (GLMM, $\beta = -0.58$, $\chi^2_1 = 4.30$, $p = 0.038$).

Wrasse were also observed intra-specifically cleaning (i.e. cleaning one another) on 19 occasions (observed in 5.0% of observations) and substrate flashing on 92 occasions (observed in 13.4% of observations). Adults most frequently intra-specifically cleaned and flashed (number of observations intra-specific cleaning seen: adults = 8, juveniles = 1, number of observations flashing seen, adults = 18, juveniles = 6). Cleaners were not more likely to perform these behaviours when they cleaned less (frequency and duration) or associated less with *I. palifera* (duration and peck tendency, exact permutation test and Pearson's χ^2 tests: all $p > 0.05$ before any multiple testing corrections).

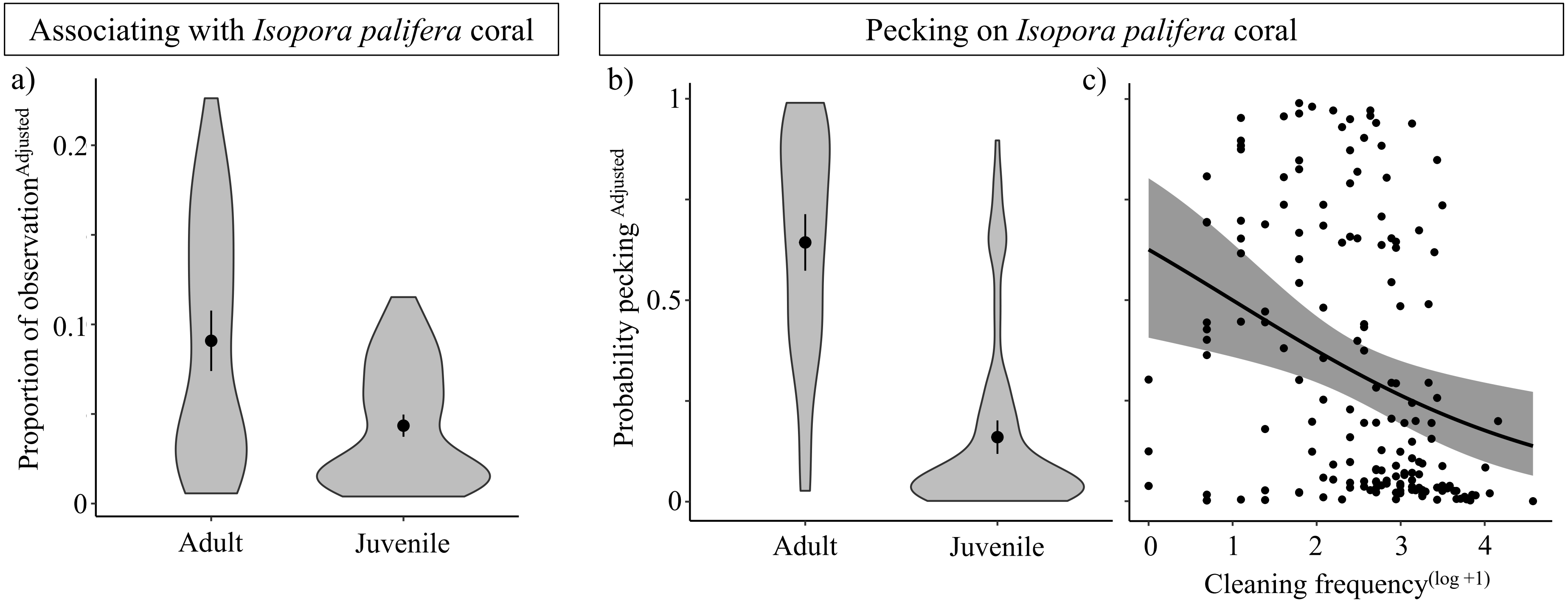


Figure 4.4: Adult and juvenile bluestreak wrasse (*Labroides dimidiatus*) associated with and pecked on the coral *Isopora palifera* within a naturally stressed tidal lagoon. The shape of the violin plot shows the distribution of adjusted model response values for (a) the proportion of time associating with *I. palifera* (GAMLSS) and (b) the pecking probability on *I. palifera* (GLMM), for juveniles and adults. Shape thickness represents the frequency of these data points occurring for each proportion/probability. Point and lines show mean ± 95% CI. c) Shows the likelihood of *I. palifera* pecking negatively correlating with cleaning frequencies (adjusted response values shown from GAMLSS). Cleaning frequencies were log (+1) transformed for clarity. A binomial smoothing term was specified to show the relationship between pecking and cleaning frequency (± S.E).

DISCUSSION

In a naturally stressed environment – a tidal lagoon, this chapter shows that a model dedicated cleaner species the bluestreak cleaner wrasse (*Labroides dimidiatus*), acts as a facultative cleaner: adults and juveniles were observed pecking at the common coral *Isopora palifera* and cleaning frequencies decreased with coral pecking. Adult wrasse also spent more time wandering across the reef, rather than occupying fixed cleaning stations, compared to juveniles. Whilst wandering, adults spent more time cleaning compared to when at their stations. Client biting was frequently observed, with biting likelihood increasing with cleaning frequency. Juveniles were more likely to bite clients when at their stations, compared to adults. Biting was not more likely to occur when wandering, however. Together this showed that within this environment, this 'dedicated' cleaner species has, at some point, partly abandoned cleaning as a food acquisition strategy.

Bluestreak wrasse are a model species for studies on cleaning behaviour, previously reported to rely solely on client gleaned material for nutrition (Côté and Soares 2011; Vaughan et al. 2017). However, this chapter documents for the first time, this iconic cleaner also pecking on the coral *I. palifera.* Given that coral pecking likelihood increased with a reduced cleaning frequency, and was more likely in the morning (cleaning can also be more frequent in the morning, Grutter 1996a), this suggests that wrasse may be gaining a food source from the coral, with the behaviour displacing cleaning (contrasting supplemental *Thalassoma bifasciatum* cleaning, **Chapter 3**). Many reef species are observed feeding on *I. palifera* (e.g. Labridae, Cole 2009, and Chaetodontidae, Nagelkerken et al. 2009), but what the acquired food source is (e.g. coral mucus, polyps and/or periphyton), remains unknown. Worryingly, *Isopora* corals are at high risk of extinction due to bleaching (Muir et al. 2017), so what these species will feed on in more bleached environments is unclear. Cleaner wrasse coral pecking was more common in adults, and begins to mirror the ontogenetic shift in cleaning behaviour of some facultative cleaners: juveniles clean, whilst adults become more generalist and consume other food types (e.g. feeding on coral polyps by *Labrichthys unilineatus,* see Cole 2009, and sponge-feeding by *Pomacanthus paru,* see Hourigan et al. 1989). Plasticity in cleaning behaviour has also been documented for several dedicated cleaner gobies (*Elacatinus* spp.) with the frequency of their cleaning versus non-cleaning feeding relying on the occupied substrate type (coral versus sponge, White et al. 2007). Changes in the environmental context influences the magnitude of benefits each partner gains and can lead to mutualism breakdown (Frederickson 2017). Returns from mutualism to a non-mutualistic state may be commonplace, especially if the mutualists have not become too specialised (Chomicki and Renner 2017), as is the case with more generalist facultative cleaners. Dedicated cleaners regularly interact with a large diversity of reef fish (e.g. 132 client species for bluestreak wrasse, Grutter and Poulin 1998b) and adjust their cleaning behaviours towards different client identities (**Chapter 7**; Bshary and Schäffer 2002). Therefore, they too could be expected to rely less on a mutualism, as this chapter has begun to show. Within this tidal lagoon, the switch towards a more facultative cleaning strategy suggests that dedicated cleaning has, at some point, broken down in this environment: longitudinal studies of cleaning are thus more important than ever. A reduction in cleaning however may have its own costs, perhaps constraining the extent of which the interaction is abandoned. For example, by frequently interacting with clients, cleaners may benefit themselves from reduced parasite loads and immune systems specifically adapted to evade commonly encountered parasites (**Chapter 2**): parasites exposure can prime immune responses (Chaplin 2010). Part-time cleaners often visit cleaning stations or attempt to elicit cleaning from heterospecifics (**Chapter 3**; Sazima et al. 1999; Arnal et al. 2000) and here intra-specific cleaning between adult bluestreak wrasse (only previously documented once between a cleaner pair, Clague et al. 2011a) and substrate flashing (not previously documented for this species) was observed. These behaviours will help to control an individual's parasite loads, so a reduction in cleaning may increase cleaners own susceptability to parasites and disease.

Ectoparasite consumption is thought to be a less nutritionally beneficial than consuming other material (e.g. sponge, White et al. 2007, or mucus, Eckes et al. 2015) so the pervasiveness of cleaning is tightly linked with the high abundance and diversity of fish species found on coral reefs. Fish species host different ectoparasite assemblages, and hence food resources (Grutter 1994) and low cleaner abundance has been linked with fish species richness (Wagner et al. 2015). To meet nutritional requirements, dedicated cleaners must frequently clean: bluestreak wrasse consume large daily quantities of ectoparasites (e.g. up to 1200, Grutter 1996a) and this may explain why many adults of larger facultative cleaner species do not clean (bigger bodies require higher energy demands, Speakman 2005). Within tidal lagoons, the movement of fish species and their larvae is tidally limited (Thresher 1983), potentially negatively influencing the diversity and abundance of available ectoparasites to the cleaner, making cleaning less profitable (either through restricted client numbers, or by the cleaners frequently interacting with the same clients). Rather than completely abandoning cleaning altogether, by switching enitrely to coral grazing, cleaners appear to have adapted their cleaning strategy instead, maintaining the occurrence of the mutualism. Like Wilson et al. (2014), bluestreak wrasse (predominantly adults), were observed to wander across the reef. Wanderers inspected/chased clients more frequently and spent more time cleaning, compared to when at their station. Bicolour cleaner wrasse (*Labroides bicolour*) wander to find clients (Mills and Côté 2010; Oates et al. 2010), and bluestreak wrasse wandering may also represent a food searching behaviour. For bluestreak wrasse it is well documented that adults spend their time at their fixed cleaning stations waiting for clients, which increases cleaning gains compared to wandering (bicolour versus bluestreak wrasse comparison; Oates et al. 2010). However, in this more stressed environment, adults, which will have higher nutritional requirements than juveniles, adopt a riskier foraging behaviour by searching for food themselves, maintaining the occurrence of the mutualism.

Mutualists can increase the magnitude of their rewards by exploiting the system (Sachs and Simms 2006). The outcomes of cleaning lie along a continuum from mutualism to parasitism (Cheney and Côté 2005) and bluestreak wrasse regularly cheat their clients, through biting more calorific mucus (Wilson et al. 2014; Eckes et al. 2015). Despite this exploitation, mutualisms often remain beneficial through space and time (Frederickson 2017), and these cleaners do alter their mutualistic versus parasitic cleaning behaviour to ensure the mutualism is maintained (Bshary 2002a; Binning et al. 2017b). Indeed, an increased biting tendency with increased cleaning frequency was also documented here. However, juveniles were also observed cheating more frequently than adults when at their cleaning stations; an intriguing result not usually observed (contrasts Mills and Côté 2010). This parasitism may help juveniles achieve their nutritional demands in this limited environment (juveniles wandered less than adults and had less *I. palifera* on their stations). Bluestreak wrasse clients usually punish cheating adults which helps to maintain the mutualism, but the magnitude of costs associated with this juvenile cheating behaviour is unknown. However, in socially-simple environments (e.g. low cleaner and client abundance, and client species richness), cheating can be more common since cleaner reputation management should be less important under these contexts (Binning et al. 2017b).

This chapter demonstrates the shift from a dedicated cleaning mutualism to a more facultative interaction for a model cleaner, the bluestreak wrasse. In a naturally stressed tidal environment, cleaners partly abandon the mutualism showing a reduced reliance on cleaning as a food source, instead feeding off *Isopora palifera* coral. Juveniles appear to exploit the system by parasitising clients more frequently than adults. The mutualism however is only partly abandoned, and adults show plasticity in their foraging behaviour by wandering across the reef searching for food, rather than waiting at their cleaning stations for clients. Thus, there still must be some benefits acquired through cleaning, or other constrictions, which prevents this cleaner from switching completely to an alternative feeding method (which has been observed for other mutualists, Sachs and Simms 2006). What these constraints are however is unknown. Both the abiotic and biotic conditions can influence mutualism dynamics (Chamberlain et al. 2014) and this chapter suggests that this stressed environmental context might shift the nature and position of this cleaning mutualism along three different continua (outlined in **Chapter 2**): mutualism to parasitism, dedicated to facultative, and solely inter-specific cleaning to both inter- and intra-specific cleaning. Through their large number of species interactions, and removal of parasites, both dedicated and facultative cleaner species play an important role in the ecological community structure (Floeter et al. 2007; Quimbayo et al. 2018). More species adopt part-time cleaning strategies (Vaughan et al. 2017) and since a dedicated cleaner shows a reduced cleaning reliance under stressed conditions, it begs the question how pervasive and stable current facultative cleaning will be?

Chapter 5

Long-Term Cleaning Patterns of the Sharknose Goby (*Elacatinus evelynae*)

A version of this chapter has been published in Coral Reefs:

Dunkley K., Ellison, A., Mohammed, R.S., van Oosterhout, C. Whittey, K.E., Perkins, S.E. and Cable, J. (2019). Long-term cleaning patterns of the sharknose goby (Elacatinus evelynae). Coral Reefs. DOI: 10.1007/s00338-019-01778-9

ABSTRACT

Cleaning interactions, which involve a cleaner removing ectoparasites and other material from the body of a heterospecific (client), are iconic symbiotic interactions observed on coral reefs worldwide. These small cleaners play a disproportionately large role in the structuring and function of coral reefs, influencing species interaction networks, client health and biodiversity. Cleaning patterns, however, are context-dependent and highly heterogeneous, and although there is some understanding about their spatial variation, longer term temporal changes in cleaning interactions have remained understudied. Given that coral reefs are globally threatened and are currently experiencing large shifts in their biodiversity, it is vital to determine which clients are consistently most important for maintaining cleaning. Using a long-term, 8 year data set (2010 – 17) on the cleaning behaviour of the predominant Caribbean cleaner, the sharknose goby (*Elacatinus evelynae*), this chapter investigated whether cleaner fish from the same reef show consistent patterns in their interactions with client species over time. Here this study conclusively shows that cleaning behaviour is highly plastic, as no single species or family was cleaned consistently more than others, in terms of cleaning frequency and duration, across all years. Only 40% of the species were cleaned more than others and ca. one third of species were also observed experiencing inconsistent cleaning patterns across years. This study thus quantifies how dynamic cleaner-client relationships are on the same reef across years and highlights the importance of long-term data.

INTRODUCTION

Symbiotic cleaning interactions are ubiquitous on coral reefs (White et al. 2007) and involve a cleaner species removing ectoparasites and other material from the body of a heterospecific (client) host (Feder 1966). During these interactions, cleaners gain a source of food (Vaughan et al. 2017) whilst clients benefit from tactile contact and parasite removal (Grutter 1999; Clague et al. 2011b; Soares et al. 2011). Despite being small in size and not highly abundant on reefs themselves (Grutter et al. 2003; Sazima et al. 2010), cleaners play a pivotal role in the structuring and functioning of coral reefs, as they interact with a wide range of fish species on a daily basis (Floeter et al. 2007; Sazima et al. 2010; Quimbayo et al. 2018). Different reef species, however, differ in their propensity to engage in cleaning interactions (Côté et al. 1998) and the nutritional material that they host, in terms of their ectoparasite assemblages and mucus composition (Eckes et al. 2015). Thus, differing clients provide asymmetric benefits to the interaction, but it is still not clear how these asymmetries in client identity and their engagement, influences cleaner-client relationships. With rapid environmental degradation threatening coral reef communities, identifying key client species and finding consistent cleaning patterns will help further knowledge on the evolution and conservation of interacting species (Toby et al. 2010).

Many studies have attempted to capture and describe cleaner-client interaction patterns (e.g. Arnal et al. 2000; Sikkel et al. 2000; Grutter et al. 2005; Soares et al. 2008c) but cleaning patterns across studies are inconsistent. For a given cleaner species, cleaning behaviour can vary with time of day (Sazima et al. 2000), cleaning station (Whiteman and Côté 2002b) and amongst reefs (Cheney and Côté 2005). All these previous studies are, however, short-term (~ 1 year), and as yet, there are no studies describing the variation in long-term cleaning patterns from the same reef. Both abiotic and biotic contexts, which will likely influence general interaction dynamics (Bronstein 2015), are more variable across rather than within locations over time. Thus, investigating consistent patterns over time within the same location and same season should minimise some sources of this variation (e.g. seasonal and location differences in ectoparasite assemblages, Grutter 1994, and general client species diversity, i.e. some species are consistently found on one reef but not another, Malcolm et al. 2007). Long-term studies are fundamental for understanding true species interaction patterns (Brown et al. 2001), and have already advanced our knowledge on coral reef communities in terms of coral decline (De'ath et al. 2012), community assemblage (Nash et al. 2016), species space use (Heupel and Simpfendorfer 2015) and species interactions (Vergés et al. 2016). Even among cleaning studies, long-term removal experiments have demonstrated the impact of cleaner presence on the health and diversity of client fish (Clague et al. 2011b; Waldie et al. 2011): a result not apparent from short-term studies (Grutter 1996b). Long-term studies on cleaner-client interactions are thus urgently required to elucidate key drivers maintaining cleaner-client interactions.

Dedicated cleaners, which are thought to rely solely on client-gleaned material for nutrition (as opposed to the opportunistic facultative cleaners, Vaughan et al. 2017), are highly connected within reef interaction networks (Quimbayo et al. 2018). The bluestreak cleaner wrasse (*Labroides dimidiatus*) and cleaning gobies (*Elacatinus* spp. formally *Gobiosoma* spp.) are the most ubiquitous and widely studied dedicated cleaner fish (Côté and Soares 2011) and can interact with a large diversity of client species daily (e.g. *Elacatinus figaro* 27 client species, Sazima et al. 2000, *Labroides dimidiatus* 132 client species, Grutter and Poulin 1998b). Gobies are the predominant cleaner fish in the Caribbean and provide an ideal model system for investigating which client species are consistently the most important for cleaning: unlike the bluestreak wrasse, the cleaning strategy of gobies does not knowingly involve tactics to manipulate client behaviour (Soares et al. 2008b; Côté and Soares 2011). Both cleaning gobies and bluestreak wrasse wait at their cleaning stations (defined by topological reef features, Potts 1973a) for clients, which choose which cleaners to visit (Bshary and Schäffer 2002). Not all client visitors get cleaned, however (**Chapters 7** and **8**; Côté et al. 1998; Arnal et al. 2001), because cleaners have a choice about which of the locally available clients to interact with and for how long. Bluestreak wrasse often adopt their cleaning behaviour to clean different clients more favourably to encourage their return (Grutter and Bshary 2003). This behaviour is not observed in gobies, and clients do not punish reluctance of cleaning (Soares et al. 2008b). Patterns of goby cleaning will hence reflect true decisions to clean certain clients by the cleaner, rather than manipulative behaviours towards different clients. In addition, Caribbean reefs, as opposed to Indo-Pacific reefs, are particularly vulnerable to loss of functional diversity (Bellwood et al. 2004) and thus understanding how their diversity is shaped is of high importance.

No study has yet quantified how consistent cleaning patterns are across time and so this chapter provides the first long-term data describing cleaning interactions of sharknose gobies (*Elacatinus evelynae*) recorded from the same coral reef over 8 years. It specifically investigates whether gobies clean different client species consistently each year, to quantify how plastic cleaning interactions really are (in terms of cleaning frequencies and mean cleaning durations). This knowledge will help to explain why we still do not fully understand the true interaction dynamics of this well studied mutualism. In addition, although cleaners interact with a number of different species, if key client species can be identified which are consistently important for facilitating the occurrence of cleaning interactions, irrespective of the fluctuating abiotic and biotic context, this will further knowledge on how this important mutualism is likely to persist under future environmental conditions.

METHODS

Cleaning interactions were observed over 8 years on Booby Reef situated in the Man O' War Bay, Tobago (11°19.344′N 060°33.484′W). This relatively degraded fringing reef begins at the shoreline and extends to Booby Island, located 85 – 90 m northeast from the shore (Ramsaroop 1982). The nearshore study area (1 – 2 m deep covering an area of 70 m x 60 m) is primarily composed of algae-covered dead coral, living brain corals (Faviidae) and an encrusting zooxanthid (*Palythoa caribaeorum*), which provides a suitable habitat for sharknose gobies (*Elacatinus evelynae*) (Soares et al. 2008c). Across 8 years no significant changes in the coral structures or reef health were observed. Tobago is situated outside of the Caribbean hurricane belt and so during this time period was unaffected by yearly reef structure damage (Gardner et al. 2005). Sharknose gobies show site fidelity to their coral cleaning stations (Whiteman and Côté 2002b), which were marked each year and matched between years using photographs (total number stations matched across 8 years = 82). Individual stations were located at least 1 m apart from one another. Within each year, not all marked stations were occupied by sharknose gobies; the number of occupied versus marked stations ranged from 79.7% in 2015 to 95.3% in 2016. Individual sharknose gobies have high turnover rates on their cleaning stations (mean age < 50 days documented in White et al. 2007) and thus different individuals will have been observed at the same cleaning stations across years. In addition, the number of gobies occupying each station, within years, ranged from one to nine (mean ± S.E. number individuals per station across years = 1.53 ± 0.01). There is no means to naturally identify individual gobies *in situ*, and thus the cleaning behaviour of different individuals will have also been observed at the same station within each year. Therefore, this study represents the selective pressures of clients in seeking out cleaning over the years, irrespective of which cleaning goby individuals are occupying the station.

Cleaning interactions were observed using snorkelling over a two week (2010 – 2015; June) or six week (2016 – 2017; May/June/July) period between the hours of 07:30 to 17:00. Focal sharknose gobies were randomly selected from marked stations for each observation and were observed for 10 minutes (2010 n = 130 observations, 2011 n = 374, 2012 n = 281, 2013 n = 143, 2014 n = 175, 2015 n = 262, 2016 n = 307, 2017 n = 304). Observations were carried out randomly across all the marked occupied cleaning stations within each year (mean ± S.E. number of observations per station; 2010 = 3.73 ± 0.54, 2011 = 8.34 ± 0.68, 2012 = 7.13 ± 0.65, 2013 = 5.56 ± 0.48, 2014 = 5.8 ± 0.77, 2015 = 6.12 ± 0.68, 2016 = 4.94 ± 0.41 2017 = 4.54 ± 0.36). During each observation, the cleaning durations and frequencies of different client species were recorded. These measures were used to calculate two probabilities of cleaning for each species in a given year using total cleaning frequencies/durations across all species and the number of species cleaned with each year. This created a standardised cleaning measures for each species, making them comparable within and across years. Five damselfish species, the dusky (*Stegastes adustus*), longfin (*S. diencaeus*), beaugregory (*S. leucostictus*), threespot (*S. planifrons*) and cocoa (*S. variabilis*) are morphologically similar and hence difficult to quickly identify in the field. These species cleaning observations and species counts on the reef were thus combined for these five species (hereafter termed *Stegastes* spp.). Frequency data for *Stegastes* spp. was subsequently divided by five to give comparable, yet conservative, values (duration data were considered in terms of the mean time per clean and thus were not adjusted). The number of different fish species within the study area were recorded each year at the start of June using 50 minute random swim surveys (n = 19 per year) and combined with sightings of species at cleaning stations for a total species count of potential clients.

Data analysis

To investigate patterns of cleaning two measures of cleaning probability for each client within each year were calculated; cleaning frequency and mean time per clean (hereafter referred to as cleaning duration). To determine whether any client species was consistently cleaned the most across years, only client species which were cleaned in three or more years were considered. These probability values were subsequently log10 transformed to increase the data resolution, which meant very small probabilities could be distinguished between. As a measure of how frequently/long clients were cleaned across years, mean cleaning probabilities on the log10 transformed values were calculated across years for each client species and cleaning type (for frequency and duration). As a measure of consistency in cleaning behaviour across years, the relative standard error (RSE) across transformed cleaning probabilities was calculated for each client species and cleaning type (frequency and duration). The RSE (expressed as a %) is similar to the coefficient of variation (CV) but provides a measure of variability whilst accounting for the mean and sample size (some clients were cleaned in 3 years whilst others were cleaned in all 8 years).

Data were analysed using R version 3.4.3 (R Core Team 2017). Z-scores were calculated for each client and for each cleaning type (frequency and duration) and for both probability and consistency measures (log10 means and RSEs) from bootstrapped (100000 resampling) means and standard deviations. The log10 mean cleaning probabilities express whether certain clients are cleaned more than others, and clients with z-scores greater than 1.64 (based on a one-sided 95% CI) are considered to be key clients to the cleaner. The RSEs reflect whether there was significant temporal variation in cleaning activity of a client species across the years, and also here, z-scores greater than 1.64 are considered to indicate a significant inconsistency. It was not possible to use a two-tailed test to determine if some clients were ‘highly non-important to the interaction’, as it was not known whether low cleaning probabilities represent a true choice to not clean by the gobies or simply a rare occurrence of the interaction between cleaner and client. P-values were computed from these z-scores, and Goodness of fit tests were subsequently used to determine whether the distribution of key clients versus non-key clients differed from a random distribution. In addition, this method was also applied to determine whether any client families were consistently cleaned more than others. Family cleaning frequencies and summed clean durations were divided by the number of species within each family, and families were only included if they were cleaned in three or more years. Finally, Spearman’s rank correlation tests were performed to determine whether there were significant relationships between the mean log10 probability values and RSEs. Two-tailed 95% confidence intervals estimated through bootstrap resampling compared whether cleaners differed in their variability in cleaning frequencies and durations. Bootstrapped z-scores were also used to determine whether the proportion of clients cleaned versus those on the reef differed across years (significant if z-scores were greater than 1.96, based on two-tailed 95% CI).

RESULTS

Across this 8 year study in Tobago, 47 client fish species, from 17 families were cleaned by sharknose gobies, *Elacatinus evelynae* (17 – 33 species cleaned within each year, Table 5.1). The number of potential client species occupying the study area ranged from 45 – 78 across years, thus only 32 to 64% of fish species on the reef were cleaned within any given year (Figure 5.1). These proportions of reef species cleaned differed significantly across years, as the percentage of clients cleaned was significantly higher in 2011 ($z = 6.08$, $p < 0.001$), but lower in 2013 ($z = -3.75$, $p < 0.001$) and 2016 ($z = -2.95$, $p = 0.002$) compared to other years. Eight fish species were recorded as clients in each of the 8 years, whilst nine species were only recorded as clients in one year, despite these species being present on the reef. For 23 fish species, they were present on the reef every year but were not always cleaned within each year (Table 5.1).

Table 5.1: Client species cleaned by sharknose gobies (*Elacatinus evelynae*) over 8 years on Booby Reef Man O' War Bay Tobago. Total clean frequencies and durations represent cumulative sums over 8 years. Time per clean represents total clean duration / total clean frequency (top 10 shaded in dark grey, lowest value shaded in light grey). Ranges show minimum and maximum ranks for different species across years with one being the most important client species. Rank changes highlight how fluid cleaner behaviour is towards species across years.

Family	Species	Years observed cleaned	Years observed on reef	Total clean frequency	Total clean duration (s)	Time per clean (s)	Rank range: clean frequency	Rank range: time per clean
Acanthuridae	*Acanthurus bahianus*	8	8	187	3041	16.3	2 – 14	4 – 13
	Acanthurus chirurgus	5	8	11	125	11.4	17 – 22	6 – 25
	Acanthurus coeruleus	7	8	98	2183	22.3	1 – 13	2 – 11
Aulostomidae	*Aulostomus maculatus*	5	8	17	163	9.6	15 – 19	5 – 21
Balistidae	*Melichthys niger*	1	8	1	24	24.0	23	4
Blenniidae	*Ophioblennius atlanticus*	1	5	6	55	9.2	16	18
Chaetodontidae	*Chaetodon capistratus*	8	8	29	163	5.6	9 – 22	9 – 22
	Chaetodon striatus	7	8	56	699	12.5	1 – 16	1 – 26
Haemulidae	*Haemulon carbonarium*	5	8	9	191	21.2	11 – 24	1 – 27
	Haemulon flavolineatum	8	8	96	2222	23.1	3 – 19	4 – 20
	Haemulon sciurus	2	7	2	25	12.5	21 – 24	2 – 18

	Haemulon chrysargyreum	3	8	23	233	10.1	9 – 19	6 – 16
Holocentridae	*Holocentrus adscensionis*	2	8	1	13	13.0	15 – 21	3 – 16
	Myripristis jacobus	1	7	1	5	5.0	21	18
Labridae	*Bodianus rufus*	2	8	2	40	20.0	21 – 23	7 – 10
	Halichoeres bivittatus	5	8	8	13	1.6	18 – 24	25 – 31
	Halichoeres maculipinna	7	8	26	228	8.8	9 – 19	5 – 25
	Halichoeres radiatus	6	8	18	251	13.9	7 – 24	1 – 25
	Thalassoma bifasciatum	3	8	4	15	3.8	22 – 24	25 – 25
Lutjanidae	*Lutjanus analis*	4	4	9	257	28.6	15 – 24	1 – 21
	Lutjanus synagris	1	2	3	70	23.3	15	7
	Ocyurus chrysurus	1	6	2	52	26.0	19	3
Monacanthidae	*Aluterus scriptus*	4	7	16	488	30.5	12 – 23	2 – 15
	Cantherhines macrocerus	7	8	69	1433	20.8	3 – 15	1 – 14
	Cantherhines pullus	3	8	6	144	24.0	17 – 19	1 – 24
Mullidae	*Mulloidichthys martinicus*	5	8	100	1222	12.2	4 – 11	2 – 21
	Pseudupeneus maculatus	2	6	2	10	5.0	21 – 23	21 – 24

Muraenidae	*Echidna catenata*	1	3	1	2	2.0	22	25
	Gymnothorax moringa	1	1	1	2	2.0	23	30
Pempheridae	*Pempheris schomburgkii*	1	6	2	24	12.0	17	11
Pomacentridae	*Abudefduf saxatilis*	4	8	19	256	13.5	10 – 24	9 – 28
	Abudefduf taurus	2	8	2	62	31.0	19 – 24	5 – 19
	Microspathodon chrysurus	8	8	113	1205	10.7	1 – 18	8 – 24
	Stegastes partitus	6	8	70	394	5.6	4 – 15	5 – 27
	Stegastes spp.[a]	8	8	1176 (235.2)	5903 (1180.6)	5.0	2 – 11	14 – 26
Scaridae	*Scarus guacamaia*	2	6	3	11	3.7	17 – 23	21 – 29
	Scarus iseri	8	8	166	1246	7.5	1 – 13	11 – 23
	Scarus taeniopterus	8	8	144	1766	12.3	3 – 12	8 – 17
	Scarus vetula	8	8	240	2405	10.0	1 – 10	6 – 16
	Sparisoma aurofrenatum	8	8	238	2892	12.2	1 – 15	5 – 17
	Sparisoma chrysopterum	2	5	2	19	9.5	22 – 23	9 – 21
	Sparisoma rubripinne	7	8	26	399	15.3	8 – 21	1 – 28
	Sparisoma viride	8	8	122	2274	18.6	1 – 15	4 – 16

Serranidae	*Cephalopholis cruentata*	3	3	7	250	35.7	15 – 18	2 – 12
	Cephalopholis fulva	1	2	4	28	7.0	18	19
	Epinephelus adscensionis	2	2	9	283	31.4	15 – 15	1 – 8
Tetradontidae	*Canthigaster valentini*	4	7	5	53	10.6	9 – 24	2 – 26

[a] *Stegastes* spp. represents five different damselfish species: *Stegastes adustus, S. diencaeus*, *S. leucostictus*, *S. planifrons* and *S. variabilis*. Total counts are provided for this group, whilst bracket values indicate these totals divided by five to act as a conservative comparable measure.

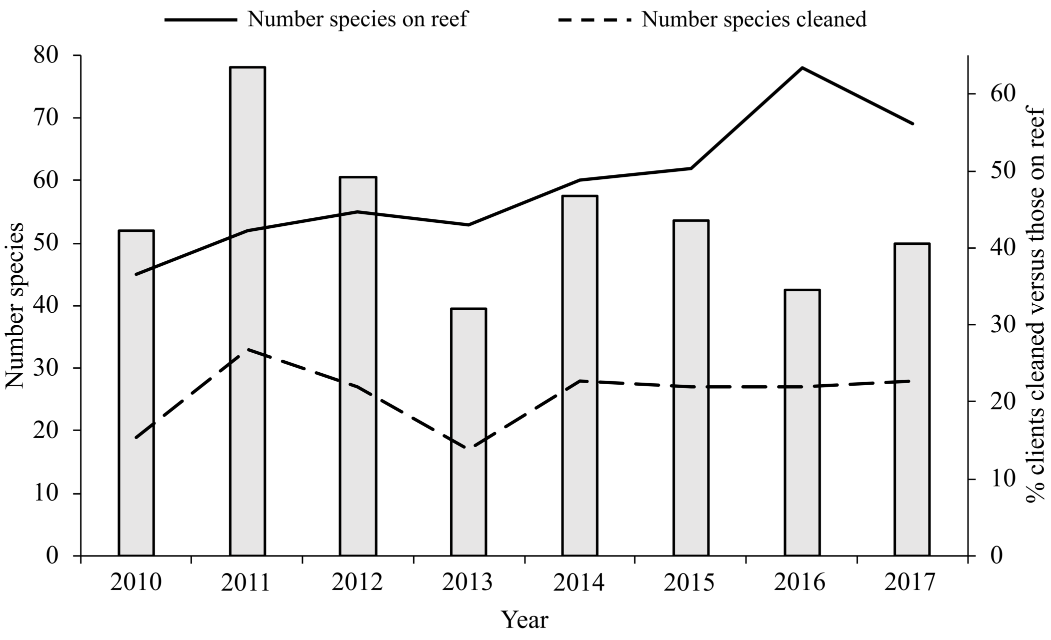


Figure 5.1: Relationship between the number of fish species on Booby Reef, Man O' War Bay Tobago and those cleaned by sharknose gobies (*Elacatinus evelynae*) over 8 years. Bars represent proportion of clients cleaned versus those available on the reef.

This 8 year study included 312 hours, 23 minutes and 22 seconds of observations at cleaning stations, within which 3154 cleaning interactions totalling 9 hours, 7 minutes and 57 seconds were observed. On cumulative values across all years, Queen parrotfish (*Scarus vetula*) were the most frequently cleaned clients, whilst five species were only observed being cleaned once. Despite being cleaned more frequently, *S. vetula* did not receive the longest cleaning duration, instead graysbys (*Cephalopholis cruentata*) were cleaned for the longest, whilst slippery dicks (*Halichoeres bivittatus*) received the shortest cleaning durations (Table 5.1). On cumulative values across 8 years, there was no significant relationship between cleaning frequencies and durations (Spearman's rho = 0.08).

Cleaners interacted with some client species significantly more than others, both in terms of their cleaning frequencies (Goodness of Fit, $\chi^2_1 = 106.04$, $p < 0.001$) and cleaning durations (Goodness of Fit, $\chi^2_1 = 112.32$, $p < 0.001$) within each year. However, across years, no single client species was consistently cleaned the most frequently, with the exception of the redband parrotfish (*Sparisoma aurofrenatum*, most frequently cleaned client in 2015 and 2016). Fourteen client species were cleaned significantly more frequently than the other species (Figure 5.2, species names highlighted in bold, from $z = 2.23$, $p = 0.013$ to $z = 9.27$, $p < 0.001$). The cleaning frequency of nine client species was significantly inconsistent across years (Figure 5.2, from $z = 2.35$, $p = 0.008$ to $z = 12.00$, $p < 0.001$). Interestingly, clients that show significantly inconsistent cleaning activity were most frequently cleaned across years, as evidenced by the positive correlation between mean log10 p-value and RSEs (Spearman's rho = 0.90, $p < 0.001$).

The client species with the highest cleaning duration differed between years (Figure 5.2). Eleven species (from $z = 2.20$, $p = 0.014$ to $z = 7.44$, $p < 0.001$) were cleaned for significantly longer than the remaining 17 clients (Figure 5.2, species names highlighted in bold). Eleven species were cleaned for significantly different durations over the 8 years (Figure 5.2, from $z = 2.43$, $p = 0.007$ to $z = 10.71$, $p < 0.001$), and again, clients that showed significantly inconsistent cleaning activity were cleaned for longer (Spearman's rho = 0.59, $p < 0.001$). Clients which were cleaned for longer were not cleaned at a higher frequency (Spearman's rho = 0.18).

Cleaners also interacted more with some client families over others, in terms of their cleaning frequencies (Goodness of Fit, $\chi^2_1 = 35.56$, $p < 0.001$) and cleaning durations (Goodness of Fit, $\chi^2_1 = 25.81$, $p < 0.001$) within each year. In seven out the eight years, Pomacentridae clients were the most frequently cleaned. In 2014, they dropped to 5th however, and Acanthuridae clients were cleaned most frequently. Four families, the Acanthuridae ($z = 4.65$, $p < 0.001$), Mullidae ($z = 2.61$, $p = 0.005$), Pomacentridae ($z = 4.70$, $p < 0.001$) and Scaridae ($z = 5.09$, $p < 0.001$) were cleaned significantly more frequently than the other nine families (Figure 5.3). Clients from three of these four families (Mullidae $z = 1.82$, $p = 0.033$, Pomacentridae $z = 5.45$, $p < 0.001$ and Scaridae $z = 8.17$, $p < 0.001$) however, were not consistently cleaned at a high frequency across all years (Figure 5.3).

For cleaning durations, no single family was consistently cleaned for longer across years, although Monacanthidae were cleaned for the longest durations in 2012 and 2015. Four families, the Acanthuridae ($z = 4.92$, $p < 0.001$), Haemulidae ($z = 2.70$, $p = 0.003$), Scaridae ($z = 1.81$, $p = 0.033$) and the Serranidae ($z = 4.92$, $p < 0.001$) were cleaned for significantly longer than the other eight families (Figure 5.3). Three families (Acanthuridae $z = 4.84$, $p < 0.001$, Lutjanidae $z = 1.97$, $p = 0.024$ and Serranidae $z = 8.64$, $p < 0.001$) did not receive the same cleaning durations across years (Figure 5.3) and those families which were cleaned for longer were not cleaned for the same duration each year (Spearman's rho = 0.66, $p = 0.022$). The cleaning frequency of a given family was unrelated to its cleaning duration (Spearman's rho = 0.03). Patterns in cleaning frequencies and cleaning durations were not more consistent for clients grouped at a family versus species level (cleaning frequency: difference between means = -2.75, 95% $\text{CI}_{\text{bootstrap}}$ [-4.06, -1.46]).

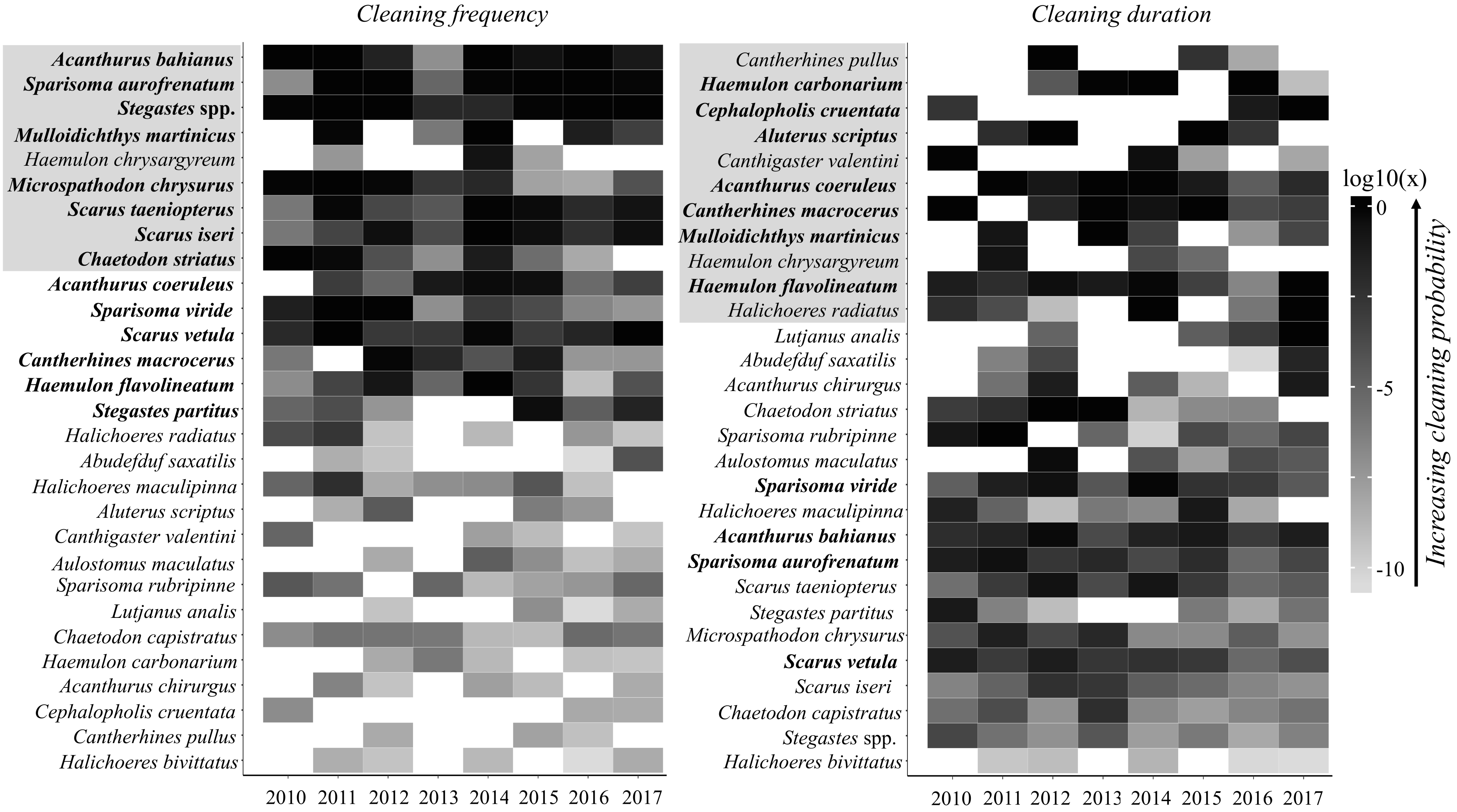

Cleaning frequency
Acanthurus bahianus
Sparisoma aurofrenatum
Stegastes spp.
Mulloidichthys martinicus
Haemulon chrysargyreum
Microspathodon chrysurus
Scarus taeniopterus
Scarus iseri
Chaetodon striatus
Acanthurus coeruleus
Sparisoma viride
Scarus vetula
Cantherhines macrocerus
Haemulon flavolineatum
Stegastes partitus
Halichoeres radiatus
Abudefduf saxatilis
Halichoeres maculipinna
Aluterus scriptus
Canthigaster valentini
Aulostomus maculatus
Sparisoma rubripinne
Lutjanus analis
Chaetodon capistratus
Haemulon carbonarium
Acanthurus chirurgus
Cephalopholis cruentata
Cantherhines pullus
Halichoeres bivittatus
2010
2011
2012
2013
2014
2015
2016
2017
Cleaning duration
Cantherhines pullus
Haemulon carbonarium
Cephalopholis cruentata
Aluterus scriptus
Canthigaster valentini
Acanthurus coeruleus
Cantherhines macrocerus
Mulloidichthys martinicus
Haemulon chrysargyreum
Haemulon flavolineatum
Halichoeres radiatus
Lutjanus analis
Abudefduf saxatilis
Acanthurus chirurgus
Chaetodon striatus
Sparisoma rubripinne
Aulostomus maculatus
Sparisoma viride
Halichoeres maculipinna
Acanthurus bahianus
Sparisoma aurofrenatum
Scarus taeniopterus
Stegastes partitus
Microspathodon chrysurus
Scarus vetula
Scarus iseri
Chaetodon capistratus
Stegastes spp.
Halichoeres bivittatus
2010
2011
2012
2013
2014
2015
2016
2017
log10(x)
0
-5
-10
Increasing cleaning probability

Figure 5.2: Sharknose goby (*Elacatinus evelynae*) probabilities of cleaning (log10 transformed) for different client species over 8 years based on observed cleaning frequencies and the mean time per clean (cleaning duration). Darker colours represent higher probabilities of cleaning. Species are ordered based on their relative standard error (RSE) of cleaning frequency or duration calculated across years, with top species showing more variation in their received cleaning behaviour across years. The names of the species that show significant ($p < 0.05$) variations in cleaning probabilities across years are shown in the grey boxes. Species that were cleaned significantly more frequently/for longer than the others are highlighted in bold.

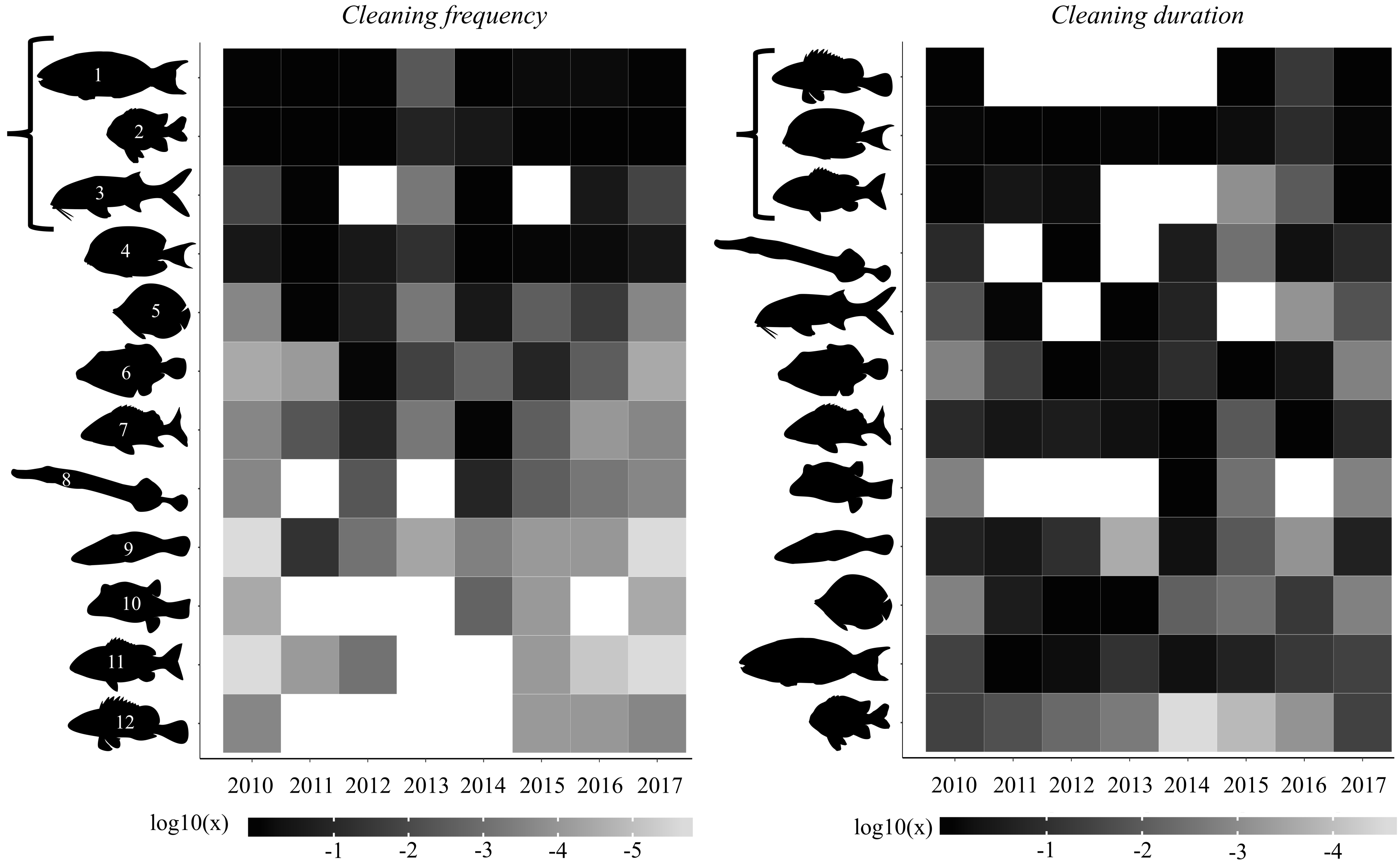

Cleaning frequency
Cleaning duration
1
2
3
4
5
6
7
8
9
10
11
12
2010
2011
2012
2013
2014
2015
2016
2017
log10(x)
-1
-2
-3
-4
-5

Figure 5.3: Sharknose goby (*Elacatinus evelynae*) probabilities of cleaning (log10 transformed) for different client families over 8 years based on observed cleaning frequencies and the mean time per clean (cleaning duration). Darker colours represent higher probabilities of cleaning. Families are ordered based on their relative standard error (RSE) of cleaning frequency or duration calculated across years, with top species showing more variation in their received cleaning behaviour across years. Those families showing significant ($p < 0.05$) variations in cleaning probabilities across years are shown using brackets. Only those families which were cleaned across 3 or more years are shown. 1= Scaridae, 2 = Pomacentridae, 3 = Mullidae, 4 = Acanthuridae, 5 = Chaetodontidae, 6 = Monacanthidae, 7 = Haemulidae, 8 = Aulostomidae, 9 = Labridae, 10 = Tetraodontidae, 11 = Lutjanidae, 12 = Serranidae.

DISCUSSION

From this unique 8 year long-term study of cleaning on a Caribbean reef, this chapter show that there are no clear patterns in cleaning frequencies and durations of particular clients across years i.e. no reef fish species is consistently the most important client of sharknose gobies (*Elacatinus evelynae*). Only around 40% of cleaned species were consistently cleaned relative to others across each year and ca. one third of species were cleaned for consistently the same duration or at the same frequency across years. Given that no client species alone appears to play a consistently key role in in maintaining cleaner-client interactions, key players in cleaner-client relationships are likely to be context dependent, and so patterns are a function of the dynamic nature of coral reef fish communities.

Cleaners are hypothesised to be supergeneralists; interacting with, and relying on a highly diverse number of species (Sazima et al. 2010). Here this study provides evidence for this notion showing that sharknose gobies do not consistently interact more with a single client species. Instead a number of species from different families were key clients for these cleaners, suggesting that a number of different client species play an important role in maintaining these cleaning interactions. Within families, species exhibit similar traits as a result of relatedness, and some previously described cleaning patterns have been confounded by phylogenetic artefacts (e.g. Grutter and Poulin 1998b; Barbu et al. 2011), although this has not always been the case (e.g. Soares et al. 2008b). Here no difference in cleaning variations towards species versus their family groupings were found, likely as a result of the diversity and number of client species and families interacting with the cleaners (Guimaraes et al. 2007). Dedicated cleaners, like the sharknose goby, are thought to rely solely on client gleaned material for nutrition (Vaughan et al. 2017) and clients are asymmetric in the nutritional content they host (Eckes et al. 2015): different client species differ in the abundance and diversity of ectoparasite species on their bodies (Grutter 1994). Thus, the presence of different species will represent different food rewards and hence nutritional gains to the cleaner. For example, larger (Poulin and Rohde 1997), group living and/or sedentary (Patterson and Ruckstuhl 2013) species, including those from the Acanthuridae, Haemulidae and Scaridae families, are likely to host greater numbers of ectoparasites/higher quality food resources relative to other clients. This may explain why they were regularly cleaned for longer than others. Ascertaining which client traits are important for cleaning has been a focus of previous literature, but again results have been inconsistent. For example, client body size has been shown to influence cleaner behaviour in some studies (e.g. Whiteman and Côté 2002a; Grutter et al. 2005; Silvano et al. 2012), but not in others (e.g. Grutter and Poulin 1998b; Arnal et al. 2000). The payoffs of certain traits will ultimately depend upon the presence, and relative abundance of both clients and their ectoparasites within the environment (Cheney and Côté 2005), which will, however, vary temporally. Thus when investigating which client traits are more important to a cleaner, the client and parasite relative abundances within an area should also be considered (Floeter et al. 2007). If we can determine which, if any, client traits are consistently important to a cleaner and always influence the interaction dynamics, we can gain a greater understanding of how cleaning mutualisms are maintained and function (see **Chapter 6**).

Previous studies have demonstrated the positive role of client abundance on the reef on cleaning frequencies (e.g. Floeter et al. 2007) but only few consider the local abundance (**Chapters 3** and **6**; Côté and Molloy 2003). Clients visit cleaners at their stations, and thus their local abundance is perhaps expected to be a larger contextual driver of cleaning patterns than the reef abundance. The local abundance of a client may also explain preference switching between years. Frequently cleaned sedentary clients from the families Pomacentridae (e.g. *Stegastes* spp. and *Microspathodon chrysurus*) and Haemulidae (e.g. *Haemulon flavolineatum*) for example, are often cleaned simply as a result of their proximity to cleaning stations. This has been shown for the longfin damselfish (*S. diencaeus*), which visits cleaning gobies less frequently the further away they are from a cleaning station (Cheney and Côté 2001). The presence of these sedentary species in close proximity to a cleaner ensures a frequent supply of food, but cleaning of these species likely represents repeated visits of the same few individuals. Indeed, often the same damselfish repeatedly visits the same cleaner (Arnal and Côté 1998) and individual damselfish with cleaning stations in their territories, have been shown to host fewer ectoparasites (Cheney and Côté 2001). These repeated visits from the same individual client may explain why those species/families that were cleaned more frequently or for longer were also the most variable in their cleaning patterns: food availability on the host may be limited.

Some species were on the reef every year but were not always observed as clients (e.g. *Abudefduf saxatilis*, Pomacentridae, and *Acanthurus chirurgus*, Acanthuridae). Again, these differences could reflect species-specific differences in ectoparasite abundance and diversity across years, influencing their need to seek out cleaning stations (Grutter 2001). However, only a maximum of two-thirds of fish species were cleaned by sharknose gobies in a given year. Cleaning by sharknose gobies is not the only method of parasite control observed on coral reefs (e.g. flashing, Wyman and Walterswyman 1985; Sikkel et al. 2000, and mucus production, Grutter et al. 2011) and on Booby Reef the presence of three other cleaner species were consistently observed across study years: juvenile blue-headed wrasse, juvenile Spanish hogfish (*Bodianus rufus*) and juvenile French angelfish (*Pomacanthus paru*). These cleaner species are facultative cleaners (Vaughan et al. 2017), and their client base can overlap with the dedicated cleaner species on the reef (**Chapter 3**; Johnson and Ruben 1988; Sazima et al. 1999; Quimbayo et al. 2018). The effect of co-occurring cleaner species may have large implications on species interactions patterns, and also influences the dynamics of other mutualistic interactions (see **Chapter 6**; Palmer et al. 2015).

Overall, this chapter shows that no client species was consistently the most important species for sharknose goby cleaners, showing that their cleaning behaviour is highly plastic across 8 years of study on the same reef, and is vulnerable to context-dependent changes. Despite sharknose goby cleaners representing one of the simplest cleaner-client interactions (in contrast to the bluestreak wrasse, *Labroides dimidiatus*), understanding of cleaning is still largely limited in what maintains and drives patterns of cleaning. Results here suggest that cleaner preference is not driven by fish species identities, and instead this study suggests combinations of other biotic context-dependent factors should now be considered when investigating cleaning dynamics (as suggested by Palmer et al. 2015 and as done in **Chapter 6**): partner identity (functional identity of both the cleaner and client, which will link to their ectoparasite assemblages and cleaner visitation patterns), partner abundance (both at the cleaning station and on the reef) and the presence of co-occurring third party species (both clients and cleaners). All these factors, among others, together likely influence which client species is important to a cleaner at any one time point, and this long-term data set provides a unique opportunity to determine how stable cleaning patterns are over time. By determining which factors are consistently important in governing cleaner-client interactions, in terms of who interacts with whom and how, we will gain a better understanding of how resilient these interactions will be in response to the impending monumental shifts in reef and marine ecosystems.

Chapter 6

Context-Dependency of a Mutualism

A version of this chapter is in preparation for journal submission.

ABSTRACT

Every animal on the planet is linked through mutualisms, yet the drivers governing these beneficial interactions are unknown. Using a long-term (8 year, including > 256 hours behavioural observations) data set of the interaction patterns of an iconic service-resource mutualism (the cleaner-client interaction) in the Caribbean, this chapter identified general principles of context-dependency in mutualistic outcomes. Here it is shown that the local client abundance and diversity, available to the cleaner, plays a key and consistent role in determining the frequency of this mutualistic service, whilst the identity of the resource provider is the most important and consistent predictor of how frequently resources are offered. Thus, the occurrence of mutualistic interactions will heavily depend on the diversity of resource providers within an environment. With biodiversity loss threatening all environments, mutualisms may breakdown, ultimately impeding key ecosystem services, such as parasite control and pollination.

INTRODUCTION

Every animal on the planet is directly or indirectly engaged in some form of mutualistic interaction (Bronstein et al. 2004). These interactions, which involve cooperation between species, are core drivers in shaping communities and have played a central role in ecological and evolutionary processes (Bronstein 2015). Despite the importance of mutualisms, we still do not understand the extent of observed spatial (Herrera 1988; Cheney and Côté 2005) and temporal (Herrera 1988; Billick and Tonkel 2003) variations and the heterogeneous interaction pattern. This has led to the hypothesis that mutualisms are context-dependent (Chamberlain et al. 2014; Hoeksema and Bruna 2015), but the biotic and abiotic context (Chamberlain et al. 2014) which favour mutualistic interactions are unknown (Hoeksema and Bruna 2000). With large environmental shifts threatening most ecosystems (Wilson 1989), it is vital to understand the underlying dynamics of an interaction which engages all of the animal kingdom.

Many mutualisms are service-resource interactions (Holland et al. 2005) where a food resource (e.g. nectar, ectoparasites or honeydew) is traded for a beneficial act (e.g. pollination, Landry 2012, parasite removal, Arnal et al. 2001 or myrmecophily, Breton and Addicott 1992). These mutualisms thus provide key ecosystem services. At their simplest level, mutualisms involve one individual interacting with another, but over time, mutualists can interact with a large number of species (Sazima et al. 2010). Who interacts with whom and how ultimately depends on how valuable the partners are to each other, since partners differ in the quality (Grutter 1994; Arnal et al. 2001; Gonzalez-Teuber and Heil 2009; Eckes et al. 2015) and quantity (Grutter 1994; Poulin and Rohde 1997; Soares et al. 2008a; Patterson and Ruckstuhl 2013) of material they host or trade. Asymmetric partners hence lead to asymmetric interaction outcomes. Within an environment, partners also vary in their abundance, the supply and demand of which will determine the value of the exchanged goods and services (Noë and Hammerstein 1994), again influencing who interacts with whom and how. A decrease in the abundance of one partner, for example, could be detrimental to the other, or facilitate a shift to a different partner (Hoeksema and Bruna 2000; Floeter et al. 2007). Ultimately changes in partner abundance will significantly influence the structure of mutualistic interaction networks (Toby et al. 2010) by altering the relative costs and benefits of the partners that are available. But, the presence of third-party species should also be considered, i.e., those in the community that are external, but available, to the focal mutualism at a specific time point (adapted from Bronstein and Barbosa 2002). These third-party species may form an important context that could modify the abundance or behaviour of a partner, altering the value of exchanged goods and services (Werner 1992). Such changes may ultimately re-wire mutualistic patterns and networks (Palmer et al. 2008; Petanidou et al. 2008), directly or indirectly harming or benefiting the participating species (Werner 1992). The combined effect of three key contextual factors; partner identity, partner abundance and the presence of third-party species, could thus impact mutualistic outcomes. What is not currently clear is how partner diversity alters mutualistic patterns spatio-temporally, and thus it is unknown how mutualistic patterns are likely to shift along species diversity gradients (Hoeksema and Bruna 2015; Palmer et al. 2015).

Partner choice is a driving force behind mutualism evolution (Noë 2001) since a strong preference for one partner will screen out others (Palmer et al. 2015). Many studies of mutualism dynamics focus on plant-pollinator interactions (Bronstein 1994; 2015), in which the pollinator selects the static, non-mobile plant. In more complex, less well studied interactions however, both partners can make choices of whom to interact with and how, especially when both are motile. Perhaps, the best-known example is the cleaner-client interactions, which are ubiquitous on coral reefs (Leung and Poulin 2008). A cleaner removes ectoparasites and debris from the body of another other species (known as clients, Feder 1966), and on one reef alone multiple species can act as cleaners and interact with a large proportion of the reef fish (Floeter et al. 2007; Sazima et al. 2010). Both individual clients and cleaners can select who to interact with, and these choices and subsequent interactions, can influence future interactions (**Chapters 7** and **8**; Bshary and Schäffer 2002; Tebbich et al. 2002). What is not clear is whether the same contextual factors that predict one partner's choice are just as important for the other (Hoeksema and Bruna 2015).

Long-term studies are key for understanding species interaction patterns (Brown et al. 2001). Using data collected over 8 years on the cleaning behaviour (256 hours and 30 minutes of behavioural observations) of the predominant Caribbean cleaner, the sharknose goby (*Elacatinus evelynae*), this chapter investigated the context-dependency of mutualistic interaction outcomes. These cleaner fish occupy cleaning stations on coral reefs which are visited by their clients (Arnal et al. 2001) and across 8 years, cleaner-client interactions at the same 82 cleaning stations on the same reef were recorded. First, this chapter quantifies the variability in cleaner and client behaviour (in terms of their cleaning and posing frequencies and durations) over reef space and time. Next, contextual factors, relating to partner identity, partner abundance and third-party species, which had the most substantial and consistent effects on cleaning interaction outcomes were identified. Clients can solicit cleaning through presenting their body to cleaners (termed posing, Feder 1966) and so finally, it was determined whether the same contextual factors also predicted clients engagement in cleaning interactions. Overall, the aim was to identify general principles of context-dependency in mutualisms to help clarify unexplained variations in interaction outcomes.

METHODS

Long-term study site

This 8 year long term study took place on the fringing shallow reef (1 – 2 m water depth) area (70 m x 60 m) of Booby Reef, Man O' War Bay Tobago (11°19.344′N 060°33.484′W, see **Chapter 5** for more detailed site description). Sharknose gobies (*Elacatinus evelynae*) show site fidelity to their brain coral cleaning stations (Whiteman and Côté 2002b), and stations were marked each year and matched between years (total number long-term stations across 8 years = 82, see Table 6.1 for within year sample sizes). Long-term stations were defined as those which were occupied by a cleaner in at least two different years. The location of each station on the reef was mapped using GPS in 2018 and individual stations were located at least 1 m apart from one another. Within each year, not all marked stations were occupied by sharknose gobies (Table 6.1 shows number long-term stations occupied out of 82). Individual sharknose gobies have high turnover rates on their cleaning stations (mean age < 50 days documented in White et al. 2007) and thus different individuals will have been observed at the same cleaning stations across years. The number of gobies occupying each station, within years, ranged from one to nine (Supplementary Table 6.1). It is not possible to naturally identify individual gobies *in situ*, and thus the cleaning behaviour of different individuals will have also been observed at the same station within each year. This study thus represents cleaner-client interaction patterns over the years, irrespective of which cleaning goby individuals are occupying the station.

Quantifying cleaner-client interactions

Cleaning interactions were observed each year using snorkelling over a two week (2010 – 2015; June) or six week (2016 – 2017; May/June/July) period between the hours of 07:30 to 17:00 (total number of observations across years = 1539). The identity, duration and frequency of cleaning of, and posing by, client species during each observation was recorded as a measure of cleaner-client behaviour. Posing involves a client presenting their body to the focal cleaner (Feder 1966). Where multiple cleaners were observed on one station, a focal individual was randomly selected for each observation (Table 6.1 for sample size) and was observed for 10 minutes.

Table 6.1: Number of occupied sharknose goby (*Elacatinus evelynae*) cleaning stations on Booby Reef, Man O' War Bay Tobago over 8 years of long-term study. Multiple 10 minute cleaner-client observations were carried out at each occupied station.

Year	Number occupied long-term stations	Total number cleaning observations	Mean (± standard error) number of observations per station
2010	15	61	4.07 ± 0.86
2011	32	271	8.47 ± 0.74
2012	31	233	7.52 ± 0.78
2013	21	108	5.14 ± 0.47
2014	24	143	5.96 ± 0.87
2015	22	166	7.55 ± 0.87
2016	60	290	4.83 ± 0.40
2017	59	267	4.53 ± 0.38

Defining contextual biotic factors

To identity which biotic factors are most important in governing cleaner-client interactions, data were collected on 12 additional variables which represent the categories of partner identity, partner abundance and the presence of third-party species (Figure 6.1). The presence of third-party species was defined here as the species in the community that are external, but available, to the focal mutualism at a specific time point (adapted from Bronstein and Barbosa 2002).

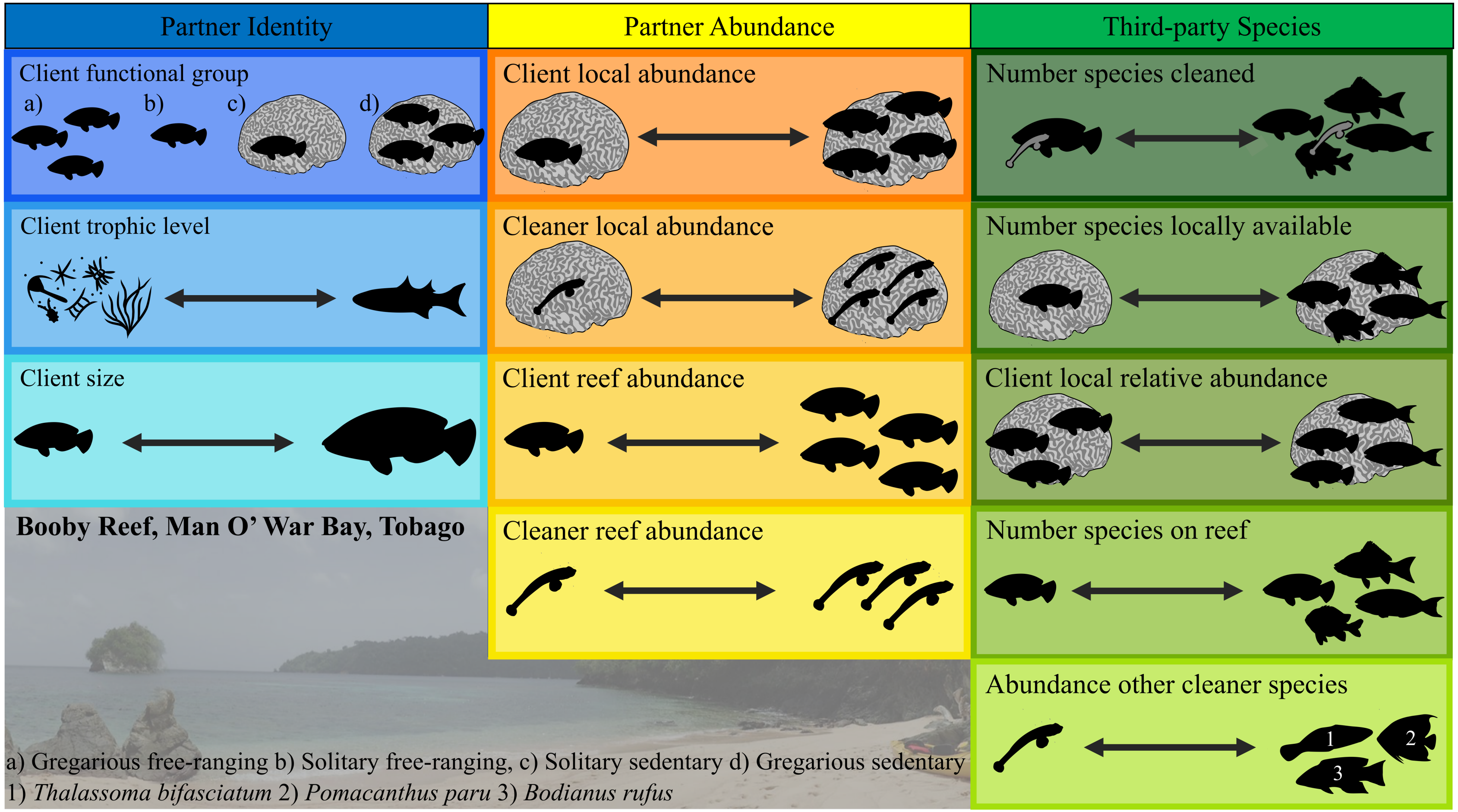


Figure 6.1: Twelve biotic contextual factors potentially driving mutualist outcomes in cleaner-client interactions from a long-term 8 year data set. Each column represents partner identity, partner abundance, and the presence of third-party species. For full details of predictors see Table 6.1 in Supplementary Materials.

I. Partner identity factors

As sharknose gobies were the focus of behavioural observations, partner identity related to the client. Each client species that was observed posing and/or being cleaned, was assigned values for their body length (max recorded fork length, Humann and Deloach 2014) and trophic level. Clients were also grouped based on their sociality (gregarious versus solitary) and mobility (free-ranging versus sedentary) behaviour on the reef (Froese and Pauly 2018, see Supplementary Table 6.1 for definitions). This meant that three contextual factors (functional group, trophic level and size) were used to represent partner identity (Figure 6.1).

II. Partner abundance factors

Abundance surveys and client data from behavioural observations were used to quantify partner abundance, which was represented by four contextual factors (Figure 6.1, see Supplementary Table 6.1 for definitions). Client reef abundance were recorded each year at the start of June using 50 minute random swim surveys (n = 19 per year), and the median numbers of fish per minute was calculated for each species which reduced the skew effect of species patchiness (e.g. shoaling behaviours). Client local abundance was quantified by recording the frequency of clients swimming by the focal cleaner (within 20 cm) during the 10 minute cleaning observations. For analyses investigating cleaning patterns, the clients' local abundance was calculated by combining posing and swimming frequencies at the station, whilst for models relating to posing, only the client's swimming frequency was used. Cleaner reef and local abundance were quantified based on the number of sharknose gobies occupying each cleaning station during randomly timed multiple presence-absence surveys, which occurred daily.

III. Presence of third-party species factors

Five factors were used to represent the presence of third-party species (Figure 6.1, see Supplementary Table 6.1 for definitions). The number of species cleaned and locally available for cleaning was quantified for each 10 minute observation: local availability represented the number of client's species posing at and/or swimming by (within 20 cm of) the cleaner (only swimming by for posing analyses). The clients' local relative abundance at the station represented the percentage of times the client species was observed posing and/or swimming by the station whilst accounting for the number of times other species interacted with the station (see Supplementary Table 6.1 for detail). The number of species on the reef was quantified by combining the diversity of species observed on abundance swims and during behavioural observations. Finally, swim surveys also provided information on the abundance of other cleaner species which were present on the reef every year (Figure 6.1, three additional part-time cleaner species present: *Bodianus rufus*, *Pomacanthus paru* and *Thalassoma bifasciatum*).

Data analysis

All analyses used Generalised Linear Mixed Models (GLMMs) which were run using lme4 (Bates et al. 2015) in R, version 3.4.3 (R Core Team 2017). Each observation across the 8 year data set (n = 1539), was assigned a unique observation ID number and all models contained the random effects of observation ID and station number. To facilitate model convergence, all continuous predictors were standardised, and models were run without using the Laplance approximation (nAGQ = 0). Observer type, which classified observers as those which collected data within a single year (n = 5) or across multiple years (n = 7), was included as a fixed effect in all models. All models were refined by removing the least significant term in each step, leaving only the best model with significant predictors. Model structures were also checked using a forward stepwise approach, to ensure that final models were not simply influenced by including a large number of predictors (Harrison et al. 2018). The significance of fixed effects was assessed using likelihood ratio tests, whilst their importance was defined by observing the proportion change in ${}_{\text{adjusted}}R^2$ that each predictor produces when it is added last to the final model or through comparing β values. Tukey's tests were used for any *post-hoc* analysis.

Cleaning and posing frequency data represent the summed interaction frequency for each client species within each observation, whilst cleaning/posing duration data represented each single cleaning/posing event and its respective interaction length. The total time for each focal observation accounted for the amount of time a cleaner was out of view, and thus varied across observations. Cleaning and posing frequencies and durations were therefore weighted by observation length. To investigate patterns in cleaning and posing frequencies a binomial model with a probit link was specified. Binomial models were checked for overdispersion. For investigating patterns in cleaning and posing durations, which represented the proportion of time each individual client spent interacting with a cleaner, a logit function was applied, and the absolute values were taken, before specifying models with Gaussian families and log links. These model structures were used for all analyses.

To test the hypothesis that cleaner-client interactions are spatio-temporally dynamic across 8 years, time of day and year were specified as fixed effects in all four GLMM models. Time of day was also nested within each year to determine whether time of day predicted cleaning and posing behaviours differently within each year. To determine whether different stations differed in their observed cleaning and posing behaviours, best fitting models with and without the random effect of station ID were compared using likelihood ratio tests. For spatial analyses, predicted values for cleaning and posing frequencies and durations using GLMM response results were calculated from final temporal models containing only significant predictors. Using these predicted values, it was then determined whether the mean values for each station (and separately their relative standard error) were spatially autocorrelated using station GPS positions and Mantel's tests. Finally, to determine whether cleaning stations that were clustered with others differed in the cleaning and posing behaviours to those that were more isolated, the presence of significant correlations between mean predicted values for each station and behaviour and their degree of aggregation were checked for. Aggregation scores were based on a PC1 value calculated from the nearest neighbour distance, the number of stations within 3 m (based on observed swimming distances of cleaners) and the number of stations within 5 m (based on maximum distance a cleaner was observed swimming from its station across the whole 8 year study).

To investigate which contextual factors are the most important for predicting mutualistic outcomes within and across years all twelve contextual factors (Figure 6.1) were specified as fixed effects and models were checked for multicollinearity using the variance inflation factor. Models were first refined based on data across all 8 years of study. The importance of each significant predictor for cleaning and posing behaviours was assessed using changes in ${}_{adj}R^2$ values when the term was added last to the model. To determine whether significant predictors of cleaning/posing frequency/duration were also significant within years, each significant predictor was nested within the categorical factor 'Year' and z-values were used to assess within year significance. When a categorical factor was significant across years, its within year significance was assessed by sub-setting the data by year and calculating likelihood ratio values.

To strengthen the within year analysis to determine how consistent contextual factors are in predicting cleaning and posing frequencies and durations, models were re-run 1,000 times with sub-sampled long-term data (n = data from 192 observations). The amount of sub-sampled data, chosen at random for each model simulation, was based on the mean number of 10 minute observations carried out within each year. This process was run for the four cleaner-client behaviours (cleaning and posing frequencies and durations). From simulated models likelihood ratio test results for the significance of each contextual factor in predicting cleaner-client behaviours were extracted, along with β coefficient values. The proportion of times that the factor significantly predicted the response variable determined which contextual factors were consistent predictors of cleaner-client behaviour. Contextual factors which significantly predicted cleaner-client behaviours in 95% of times ($\alpha = 0.05$) were considered consistent predictors, whilst those with $p > 0.05$ were defined as dynamic predictors of cleaning/posing. The β coefficient values shows the effect direction of each contextual factor (except for the categorical ‘Client functional group’: this was not an issue as ‘Client functional group’ was never a consistent predictor of cleaning or posing behaviours). To determine which consistent contextual predictors were more important in predicting cleaning/client behaviours, each consistent predictor within each model was ranked based on their absolute β value, with higher β values indicating a more important contextual factor (factors were all standardised for comparison). P-values were subsequently calculated representing the proportion of times (from 1,000 models) that each contextual factor was ranked as most important: if $p < 0.05$ it suggested that the contextual factor was not the most important, relative to others, predictor of cleaning/posing behaviour.

RESULTS

How does the mutualism differ spatio-temporally?

Cleaning and posing frequencies, and posing durations, differed between years (GLMM, clean frequency: $\chi^2_1 = 34.42$, $p < 0.001$, pose frequency: $\chi^2_1 = 78.51$, $p < 0.001$, clean duration: $\chi^2_1 = 11.17$, $p = 0.132$, pose duration: $\chi^2_1 = 22.89$, $p = 0.002$, Tukey's $p < 0.05$, see Supplementary Table 6.2). Contrasting previous studies (Sazima et al. 2000; Côté and Molloy 2003), cleaning behaviour did not differ with time of day, a pattern that was observed across all 8 years of data (GLMM, clean frequencies and durations all $p > 0.05$, Supplementary Table 6.2). Posing frequencies were however highest earlier in the day, in 3 of 8 years (pose frequency GLMM, 2010: $z = -2.31$, $p = 0.021$, 2011: $z = -3.58$, $p < 0.001$, 2013: $z = -3.43$, $p < 0.001$, pose durations GLMM, $p > 0.05$, Supplementary Table 6.2).

Spatially, cleaning behaviours and posing frequencies showed no discernible pattern across the 8 year study (Figure 6.2, LRT, clean frequency: $\chi^2_1 = 22.65$, $p < 0.001$, clean duration: $\chi^2_1 = 25.09$, $p < 0.001$, pose frequency: $\chi^2_1 = 25.13$, $p < 0.001$, pose duration: $\chi^2_1 = 0.37$, $p = 0.543$). Stations that were situated closer to one another, however, did not show similar cleaner-client interactions, compared to stations that were further apart (Figure 6.2, no spatial autocorrelation, Mantel's tests all $p > 0.100$, Supplementary Table 6.2). Similarly, stations that were clustered with other stations (i.e. considered aggregated) did not differ in their cleaner-client interaction patterns across years compared to those stations that were considered more isolated on the reef (Figure 6.2, Pearson correlation clean and pose frequencies and durations with an 'aggregation PC1 score' all $p > 0.100$, Supplementary Table 6.2).

Which contextual factors are the most important and consistent predictors of cleaner behaviour across 8 years?

Ten of the 12 biotic factors, relating to partner abundance, partner identity and the presence of third-party species (Figure 6.1), significantly predicted cleaning frequencies across 8 years of study (Table 6.2, GLMM $_{adj}R^2 = 39.2\%$). Factors relating to the presence of third-party species were together the most important predictors of cleaning frequencies, with the number of species cleaned being the most important single predictor of cleaning frequencies across years, having a positive effect (Figure 6.3, mean R^2 proportion change per factor when factor(s) added last to final model =

7.4%, partner identity = 6.3%, partner abundance = 4.8%, number of species cleaned = 27.0%).

Only four of these ten important factors, however, were consistent predictors of cleaning frequencies (Figure 6.3, proportion of 1,000 simulations significant > 95%, see Supplementary Table 6.3a). Cleaning frequencies consistently increased with the client's local abundance at the station and the number of species cleaned but decreased when the client's trophic level and the number of species locally available increases (Figure 6.3). The remaining six significant predictors can hence be considered dynamic predictors of cleaning frequencies (Figure 6.3). It was not possible to identify which of the four factors was consistently the most important predictor of cleaning frequencies but the clients' local abundance was never the most important (based on correlations between ranked absolute standardised β values within each simulation all, 95% CI for β values, Client trophic level [0.11, 0.26], Number species cleaned [0.14, 0.25], Number species locally available [0.10, 0.22] and Client local abundance [0.04, 0.15]).

Only four biotic factors predicted cleaning durations across all 8 years (Table 6.2, GLMM ${}_{adj}R^2 = 24.3\%$). Client reef abundance was the most important predictor, with decreased durations when client abundances were high (Figure 6.3, R^2 change = 5.1%). None of these factors, however, were consistent predictors of cleaning durations (Figure 6.3, proportion of 1,000 simulations significant all $p > 0.2$). This result was also reflected within years, since factors only predicted cleaning durations in a maximum of 3 of the 8 years (Figure 6.3, Supplementary Table 6.3b). After accounting for the role of biotic factors in predicting cleaning across years, cleaning stations still differed from one another in their cleaning durations (LRT, $\chi^2_1 = 15.67$, $p < 0.001$).

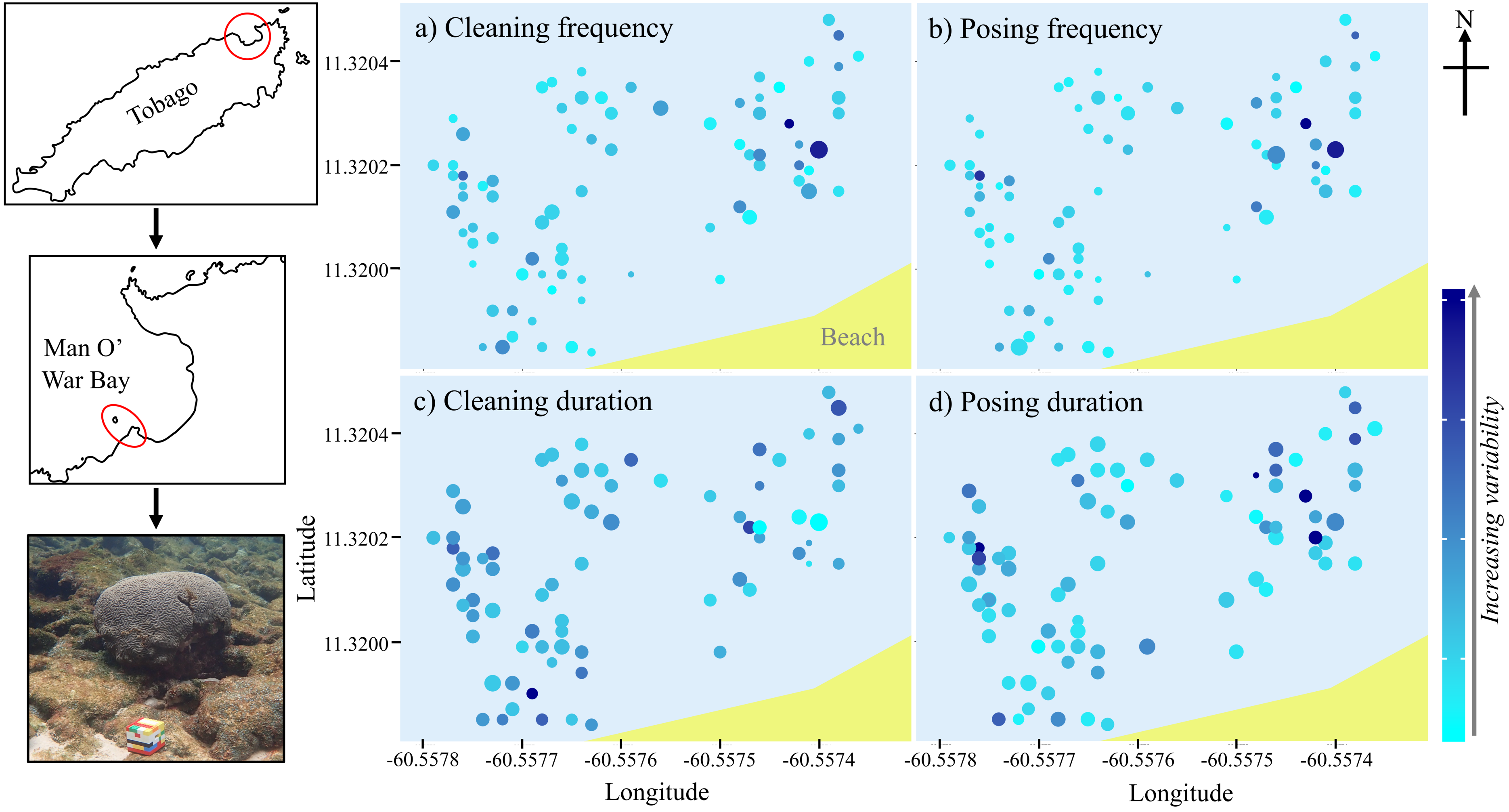

Tobago
Man O'
War Bay
a) Cleaning frequency
b) Posing frequency
c) Cleaning duration
d) Posing duration
Beach
N
Latitude
11.3204
11.3202
11.3200
Longitude
-60.5578
-60.5577
-60.5576
-60.5575
-60.5574
Increasing variability

Figure 6.2: Spatial distribution and cleaning patterns of 82 sharknose goby (*Elacatinus evelynae*) cleaning stations on Booby Reef, Man O' War Bay Tobago. Each circle represents a cleaning station and the scaled size of the circle represents mean a) cleaning frequencies, b) posing frequencies, c) cleaning durations and d) posing durations with larger circles showing increased frequencies or durations across years (predicted values from GLMMs). The colour of each circle represents the variation of this mean value and is based on the relative standard error: darker colours represent stations which were more variable in their cleaning and/or posing frequencies and/or durations across years. Photograph shows example of an isolated (no other neighbouring cleaner stations) long-term cleaning station.

Table 6.2: Significant GLMM predictors of sharknose goby (*Elacatinus evelynae*) cleaning frequencies and durations and their clients posing frequencies and durations across 8 years. Contextual factors are nested with the categories partner identity (PI), partner abundance (PA) and the presence of third-party species (TP). REL = relative abundance.

Cat	Factor	Cleaning		Posing	
		Frequency	Duration	Frequency	Duration
PI	Client functional group	$\chi^2_3 = 148.09$, $p < 0.001$	$\chi^2_3 = 13.77$, $p = 0.003$	$\chi^2_3 = 241.88$, $p < 0.001$	$\chi^2_3 = 50.00$, $p < 0.001$
	Client trophic level	$\chi^2_1 = 278.11$, $p < 0.001$	-	$\chi^2_1 = 211.05$, $p < 0.001$	$\chi^2_1 = 14.10$, $p < 0.001$
	Client size	$\chi^2_1 = 8.18$, $p = 0.004$	$\chi^2_1 = 21.44$, $p < 0.001$	$\chi^2_1 = 13.46$, $p < 0.001$	-
PA	Client local abundance	$\chi^2_1 = 214.00$, $p < 0.001$	-	$\chi^2_1 = 99.27$, $p < 0.001$	-
	Client reef abundance	-	$\chi^2_1 = 35.12$, $p < 0.001$	$\chi^2_1 = 6.02$, $p = 0.014$	-
	Cleaner local abundance	$\chi^2_1 = 4.38$, $p = 0.036$	-	$\chi^2_1 = 6.58$, $p = 0.010$	-
	Cleaner reef abundance	-	-	$\chi^2_1 = 10.50$, $p = 0.001$	-
TP	Number spp. cleaned	$\chi^2_1 = 585.22$, $p < 0.001$	-	$\chi^2_1 = 36.46$, $p < 0.001$	-
	Number spp. locally	$\chi^2_1 = 271.36$, $p < 0.001$	-	-	-
	Client local REL	$\chi^2_1 = 21.01$, $p < 0.001$	-	$\chi^2_1 = 106.66$, $p < 0.001$	-
	Number spp. reef	$\chi^2_1 = 9.53$, $p = 0.002$	$\chi^2_1 = 4.36$, $p = 0.037$	-	-
	Abundance other cleaner spp.	$\chi^2_1 = 10.58$, $p = 0.001$	-	$\chi^2_1 = 52.13$, $p < 0.001$	-

Which contextual factors are the most important and consistent predictors of client posing behaviour across 8 years?

Ten biotic factors, relating to partner abundance, partner identity and the presence of third-party species, significantly predicted posing frequencies across 8 years of study (Table 6.2, GLMM $_{adj}R^2$ = 33.1%). Posing durations were only predicted by two factors relating to partner abundance (Table 6.2, GLMM $_{adj}R^2$ = 28.7%).

Contrasting cleaning, factors relating to the clients' identity were the most important predictors of posing frequencies and durations across years (Figure 6.3, pose frequency: mean R^2 proportion change per factor, partner identity = 6.8%, partner abundance = 1.6%, third party species = 6.2%, posing duration: partner identity = 19.4%). The clients' functional group was the most important predictor of both posing frequencies and durations across years (posing frequency: R^2 change = 13.2%, posing duration: R^2 change = 5.7%): solitary free-ranging clients posed more frequently and for longer than the other three client types (Figure 6.1). After accounting for the role of biotic factors in predicting posing across years, cleaning stations still differed from one another in their posing frequencies and durations (LRT, $\chi^2_1 = 18.52$, $p < 0.001$).

Posing frequencies were only consistently predicted by the client's trophic level (Figure 6.3, proportion of 1,000 simulations significant = 100%): posing frequencies consistently decrease with increased trophic level. This result was also reflected in the within year analysis; client trophic levels predicted posing frequencies in 7 out of 8 years (Figure 6.3, Supplementary Table 6.3c, GLMM, 2012 $z = -1.85$, $p = 0.065$). Like cleaning durations, no factors across (1,000 simulated GLMMs, proportion significant $p > 0.100$, Figure 6.3) or within years (Figure 6.3, Supplementary Table 6.3d) consistently predicted posing durations.

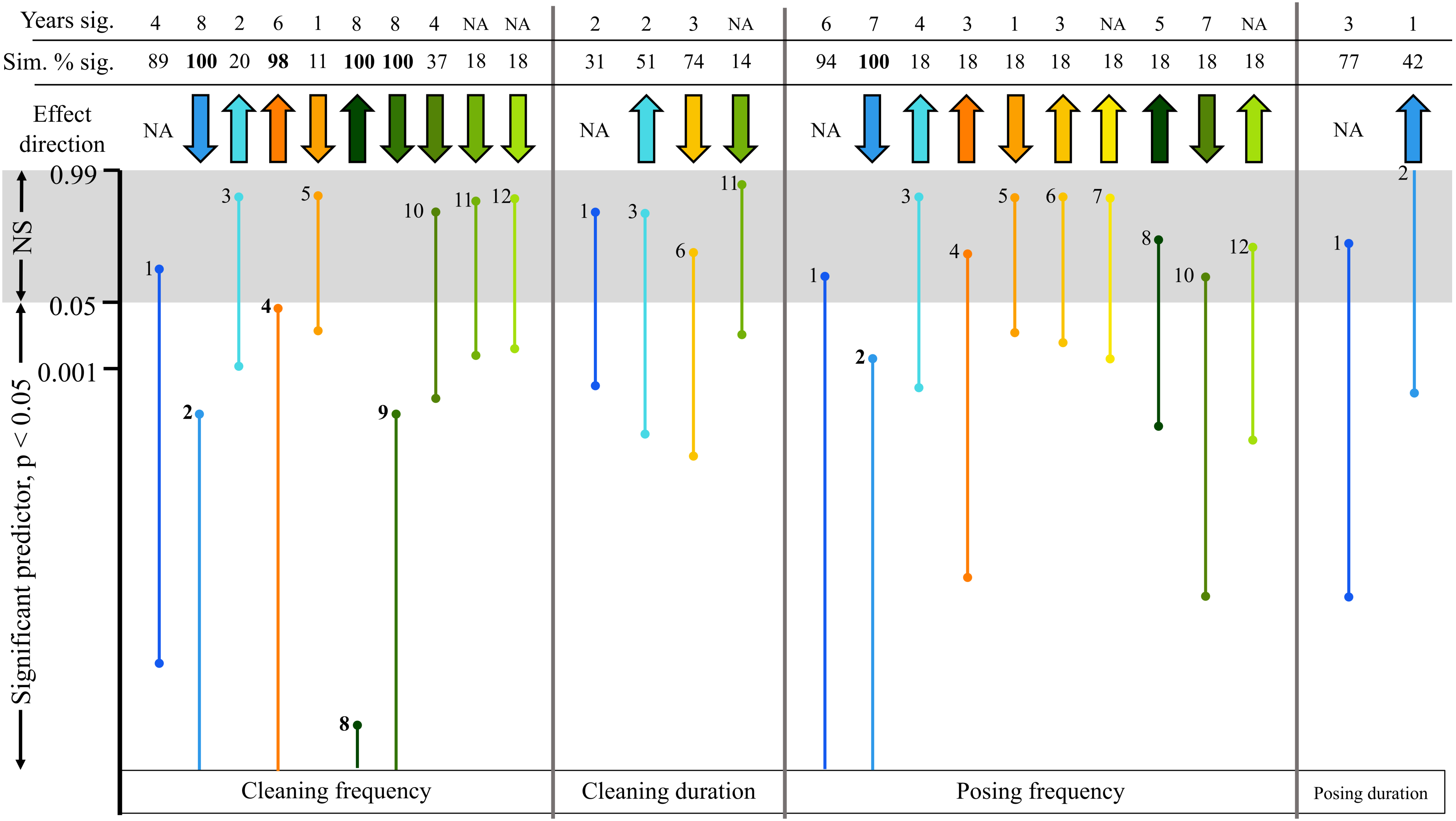

Years sig.
4 8 2 6 1 8 8 4 NA NA
2 2 3 NA
6 7 4 3 1 3 NA 5 7 NA
3 1
Sim. % sig.
89 100 20 98 11 100 100 37 18 18
31 51 74 14
94 100 18 18 18 18 18 18 18 18
77 42
Effect direction
NA
NA
NA
NA
0.99
0.05
0.001
NS
Significant predictor, p < 0.05
1 2 3 4 5 8 9 10 11 12
1 3 6 11
1 2 3 4 5 6 7 8 10 12
1 2
Cleaning frequency
Cleaning duration
Posing frequency
Posing duration

Figure 6.3: Consistent and dynamic contextual predictors of sharknose goby (*Elacatinus evelynae*) cleaning and client posing behaviour (frequencies and durations) on Booby Reef, Man O' War Bay, Tobago. From an 8 year data set of 1539 observations, random subsamples were selected (n = 192 observations per simulation) and GLMM models were re-run 1,000 times. Bar lengths show the range of generated p-values for each predictor across these simulated models, whilst 'Sim. % sig.' shows the percentage of times each predictor significantly predicted ($p < 0.05$) cleaning and/or posing behaviour (frequencies and durations) out of 1,000. P-value ranges were plotted on a logit scale while the y axis values show the position of the untransformed p-values (NS = not significant, $p > 0.05$). The years significant (sig.) represents the number of years within the data set (out of 8) the predictor was significant ($p < 0.05$) (see Supplementary Table 6.3) and the effect direction shows the positive or negative effect each predictor had on cleaner-client behaviour. Predictors are numbered from 1 – 12 (with colours matching Figure 6.1) and bold formatting represents those factors which were consistent predictors of cleaner or client behaviour. Effect directions could not be obtained for the categorical factor, client functional group, and some contextual factors did not differ within years: these values are denoted by 'NA'. 1 = client functional group, 2 = client trophic level, 3 = client size, 4 = client local abundance, 5 = cleaner local abundance, 6 = client reef abundance, 7 = cleaner reef abundance, 8 = number of species cleaned, 9 = number of species locally available, 10 = client local relative abundance, 11 = number client species on reef, and 12 = abundance of other cleaner species on reef.

DISCUSSION

For the first time, this unique long-term 8 year study on an iconic mutualism (cleaner-client) has quantified how context-dependency governs who interacts with whom and how. Here, cleaning patterns varied temporally and spatially, with different cleaning stations differing in their cleaning frequencies and durations, and the length of time clients spent posing. Contextual factors relating to the partners identity, abundance and the presence of third-party species played a significant role in predicting both cleaner and client behaviour. The client's trophic level, their local abundance at the cleaning station, the diversity of species cleaned and diversity locally available, consistently influenced cleaner-client interaction frequencies over 8 years of observations, whilst the remaining factors (client functional group and size, cleaner local and reef abundance, client reef and relative local abundance, and cleaner and client species reef diversity) played a dynamic role in predicting both the quality and quantities of interactions. This thus shows that the local, rather than reef level environment plays a pivotal role in cleaning dynamics and highlights the need to consider multiple contextual factors when investigating mutualistic patterns.

Mutualistic interactions are governed by conflicts of interest (Sachs and Simms 2006): the service provider (the cleaner) gains a food reward (Arnal et al. 2001), whilst the resource provider (the client) benefits from the service (here through parasite removal and tactile stimulation Grutter 2001; Clague et al. 2011b; Soares et al. 2011). Different drivers are thus expected to influence the cleaners versus clients' mutualistic engagement, and this chapter demonstrates for the first time, that different context types (partner identity, partner abundance and the presence of third-party species) influence the cleaners versus the client's behaviour to different degrees and are of differing importance to the interaction outcome. Cleaning behaviours were predominantly regulated by the presence of third-party species and partner abundance, whilst partner identity regulated client posing behaviours. Third-party species were defined here as those species in the community that not directly involved but available to the focal mutualism at a specific time point (adapted from Bronstein and Barbosa 2002), as also observed in plant-pollinator interactions. Dedicated cleaners, like the sharknose goby (*Elacatinus evelynae*) gain all their energy and nutrition from client derived material (Vaughan et al. 2017) and are thus expected to choose a foraging option that maximises their efficiency and hence daily energy gains (Nolet 2002).

Without breaking down the mutualistic interaction through exploitation or abandonment (Sachs and Simms 2006), higher energy gains can be obtained through consuming higher quality foods, feeding for longer and increasing diet breadth (Toscano et al. 2016). Indeed here, cleaning frequency increased with the diversity of clients cleaned (increasing diet breadth). Rarer clients were also cleaned for longer (like Arnal et al. 2000). Different clients are asymmetric in the quality and quantity of the material they host (e.g. parasites Grutter 1994; Arnal et al. 2001; Soares et al. 2008a; Eckes et al. 2015), based on their traits (e.g. predatory Lester and McVinish 2016, larger Poulin and Rohde 1997, group living and/or sedentary Patterson and Ruckstuhl 2013) or abundance (more abundant reef species visit cleaners frequently and show reduced ectoparasite loads, Cheney and Côté 2003), and thus choosing between, and interacting with different choice options would be energetically advantageous for a cleaner (increasing gains whilst minimising interaction frequency). Indeed, cleaners have been previously shown to selectively interact with clients that offer greater food rewards (**Chapters 3** and **7**; Soares et al. 2008a). There may be a constraint on increased client choice however, since here cleaning frequencies also decreased when the local and reef client diversity and abundance increased. There could be costs for an individual interacting with a higher density of other individuals since it may reduce an individual's attention for other potential threats (e.g. predators; Milinski 1984) or create a 'confusion effect' (Krakauer 1995; Ward et al. 2004) where the presence of too much choice may make it difficult for the cleaner to assess what is available (as also suggested in **Chapter 3**).

Given that parasites reduce the fitness of an animal (Dobson and Hudson 1986), client differences in parasite assemblages may also explain why partner identity was the most important predictor of client posing behaviours. For clients with traits that are prone to high parasite burdens it would be beneficial to visit cleaning stations more frequently and for longer durations, as documented here. It has been previously suggested, that species abundance determines interspecific interaction frequency and strength (Vázquez et al. 2007), but as hunger is a proximate cause of mutualisms (Côté and Molloy 2003), and species benefit from food choice options (Toscano et al. 2016), it is unsurprising that, as shown here, the presence of third-party species plays a strong role on the service providers behaviour.

Not all identified contexts however, consistently governed cleaner and client behaviours and no contextual factor measured in the current study was a consistent predictor of posing and cleaning durations. Instead interaction durations may be more consistently driven by individual level features of the partner, such as physiological state and metabolism (Toscano et al. 2016), which could influence the amount of investment by an individual in each interaction. There is also a cost for clients to visit cleaners (e.g. missed foraging opportunities, Poulin and Vickery 1995), and thus clients will be somewhat limited in how frequently or for how long, they can visit cleaners. These individual level differences may also explain why spatial differences in both cleaning and posing were found; cleaning stations differed in their observed cleaning and posing durations, which may reflect phenotypic variation in cleaner and client distributions across the reef (**Chapters 7** and **8**). In addition, the microhabitat features of stations should also be considered, since microhabitat influences the spatial distribution of reef species (Whiteman and Côté 2004b) and its direct and indirect role in influencing cleaner-client interaction patterns is currently unknown.

It is well established that mutualisms are context-dependent and dynamic interactions (Chamberlain et al. 2014; Hoeksema and Bruna 2015), and here, for the same reef, this study documented multiple contextual factors that predicted cleaner-client interactions in some instances but not others. Previous studies that have attempted to ascertain which features are important in predicting interaction patterns using only short-term data, have produced mixed results. For example, client body size influences cleaning behaviour in some studies (Whiteman and Côté 2002a; Grutter et al. 2005; Silvano et al. 2012) but not in others (Grutter and Poulin 1998b; Arnal et al. 2000). Of course, these variations are to be expected across studies, since here client body size was a dynamic contextual predictor of cleaning frequencies and durations on the same reef. Thus, the cautionary note is, that without a time series of data it is not clear which factors will be dynamic or consistent predictors of mutualisms. It is unclear why dynamic contextual factors drop in and out of importance, but it is likely due to the abiotic and biotic context of these predictors. The parasitic context for example, is likely to be a large driver of these dynamic patterns. Parasites are an important component of cleaner-client interactions (Arnal et al. 2001) and parasite abundance and diversity of any given individual can differ spatio-temporally (Grutter 1994;1996a; Côté and Molloy 2003), influencing the relative value of the client to the cleaner during each interaction. Cleaners do adjust their service quality and quantity based on the clients' available resources (Bshary and Grutter 2002b). This may explain why factors relating to available client assemblages (number species cleaned and locally available, and client abundance) were consistent predictors of cleaning frequency for example. By adopting a supergeneralist feeding behaviour, interacting with and relying on a large diversity of client species (Sazima et al. 2010), cleaning can be maintained as a stable food source through choice options, irrespective of parasite induced shifts in food availability and diversity within and across client species. The context of the contextual factor will therefore play a role in governing interactions patterns: mutualisms can hence be regulated both directly and indirectly by context-dependency.

In summary, this chapter used multiple contextual factors from a long-term data set, to predict the patterns in partner behaviour within a ubiquitous (White et al. 2007) and complex mutualistic interaction. Mutualisms involve every animal on the planet (Bronstein et al. 2004), and the effects of other species, and their respective traits, should not be neglected when investigating who interacts with whom and how. Mutualisms involve one individual interacting with another at any one time, and thus the behaviours and traits of an interacting partner will contribute to the ecological and selective environment of the other (Wolf and Weissing 2012). The presence of other species however will also consistently influence the selective environment, decisions and behaviours of the service provider influencing the interaction outcomes. The prevalence of mutualistic interactions within an environment, which have choice options (e.g. cleaner-client and plant-pollination), is thus expected to shift along diversity and abundance gradients, a harrowing observation when biodiversity is rapidly declining within our ecosystems (Balvanera et al. 2006), and especially since mutualists can drive patterns of diversity (Grutter et al. 2003; Waldie et al. 2011). Key mutualisms are not immune to breakdowns (Palmer et al. 2008), and it is not clear how ecosystem services (e.g. pollination, Landry 2012, and parasite control, Arnal et al. 2001) will function as a result.

SUPPLEMENTARY MATERIAL

Supplementary Table 6.1: Detailed descriptions of biotic contextual factors used to predict cleaning and posing behaviours across and within 8 years.

Category	Factor	Definition
Partner Identity (PI)	Client functional group	FishBase (Froese and Pauly 2018) was used to record clients as either solitary or gregarious (associate with > 3 individuals) and sedentary or free ranging.
	Client size	Client species assigned maximum fork lengths using (Humann and Deloach 2014). Range 9 – 150 cm.
	Client trophic level	Client species assigned trophic levels using FishBase (Froese and Pauly 2018). Range 2 – 4.4.
Partner Abundance (PA)	Client local abundance	Posing frequencies were combined with the frequency of clients swimming by the focal cleaner (within 20 cm).
	Client reef abundance	Median per minute values of each client species based on n = 19 (per year) 50 minute random swim surveys.
	Cleaner local abundance	Number of gobies occupying station for the observation. Range 0 – 9.
	Cleaner reef abundance	Mean number of gobies occupying the stations within each year. Range 0.6 – 1.28
	Number species cleaned	Number of different species observed being cleaned within each observation. Range 0 – 7

Presence of Third-Party Species (TP)	Number species locally	Number of different species observed posing at and/or swimming by the cleaning station within each observation. Range 0 – 14
	Number species reef	Based on fish counts at the start of June and cleaning observations. Range 45 – 78 spp.
	Client local REL	Relative abundance (REL) of clients at the station, based on ‘Client local abundance’ and the total local abundance of different species at the station. Range 0 – 1
	Abundance other three cleaner species on reef	Based on fish species counts used to identify ‘Client reef abundance’. Range 0.72 – 3.19

Supplementary Table 6.2: GLMM model outputs determining whether cleaning and posing behaviours differed spatio-temporally. Year differences are numbered 1 – 8 representing the years 2010 – 2018 and show Tukey’s *post hoc* groupings.

	Clean frequency	Clean duration	Pose frequency	Pose duration
Year	$\chi^2_1 = 34.42$, $p < 0.001$	$\chi^2_1 = 11.17$, $p = 0.132$	$\chi^2_1 = 78.51$, $p < 0.001$	$\chi^2_1 = 22.89$, $p = 0.002$
Year pattern	Lower in 2013 vs 2010, 2012, 2014, higher in 2012, 2014 vs 2016	NA	Lowest 2011, 2013	Lowest in 2013, 2015, 2017 vs 2014
Year differences	1 2 3 4 5 6 7 8 bc ac c a c ac ab ac	NA	1 2 3 4 5 6 7 8 ab a b a b b b b	1 2 3 4 5 6 7 8 ab ab ab b a b ab b
Time of day	$\chi^2_1 = 0.07$, $p = 0.786$	$\chi^2_1 = 0.05$, $p = 0.816$	$\chi^2_1 = 0.96$, $p = 0.327$	$\chi^2_1 = -0.018$, $p = 1.00$

Station differences?	$\chi^2_1 = 22.65$, $p < 0.001$	$\chi^2_1 = 25.09$, $p < 0.001$	$\chi^2_1 = 25.13$, $p < 0.001$	$\chi^2_1 = 0.37$, $p = 0.543$
Spatial patterning?	$r = -0.015$, $p = 0.650$	$r = 0.051$, $p = 0.151$	$r = 0.026$, $p = 0.249$	$r = -0.017$, $p = 0.299$
Degree of aggregation	$r_s = -0.02$, $p = 0.865$	rs = 0.06, p = 0.610	rs = 0.04, p = 0.711	rs = -0.06, p = 0.5846

Supplementary Table 6.3: Within year significance of each of the significant long-term predictors of cleaning frequencies (a), durations (b) and posing frequencies (c) and durations (d). Significant years are shaded in grey. Some variables were quantified once per year and thus did not vary within years (indicated by ‘-‘).

(a) Cleaning frequency

	2010	2011	2012	2013	2014	2015	2016	2017
Functional group	$\chi^2_3 = 4.51$, $p = 0.212$	$\chi^2_3 = 3.48$, $p = 0.323$	$\chi^2_3 = 20.20$, $p < 0.001$	$\chi^2_3 = 3.45$, $p = 0.327$	$\chi^2_3 = 58.09$, $p < 0.001$	$\chi^2_3 = 4.98$, $p = 0.174$	$\chi^2_3 = 60.51$, $p < 0.001$	$\chi^2_3 = 54.74$, $p < 0.001$
Client trophic	$z = -2.10$, $p = 0.036$	$z = -4.83$, $p < 0.001$	$z = -6.26$, $p < 0.001$	$z = -4.53$, $p < 0.001$	$z = -6.62$, $p < 0.001$	$z = -4.34$, $p < 0.001$	$z = -6.92$, $p < 0.001$	$z = -5.70$, $p < 0.001$
Client size	$z = -0.98$, $p = 0.327$	$z = -0.92$, $p = 0.356$	$z = -0.89$, $p = 0.373$	$z = 0.26$, $p = 0.798$	$z = 3.02$, $p = 0.002$	$z = 0.42$, $p = 0.674$	$z = 1.39$, $p = 0.164$	$z = 6.37$, $p < 0.001$
Client local abundance	$z = 3.54$, $p < 0.001$	$z = 2.16$, $p = 0.031$	$z = 7.56$, $p < 0.001$	$z = 0.56$, $p = 0.577$	$z = 7.16$, $p < 0.001$	$z = 4.04$, $p < 0.001$	$z = 7.39$, $p < 0.001$	$z = 1.76$, $p = 0.079$
Cleaner local abundance	$z = -1.15$, $p = 0.249$	$z = -1.34$, $p = 0.179$	$z = 0.69$, $p = 0.488$	$z = -0.64$, $p = 0.523$	$z = -0.73$, $p = 0.463$	$z = 0.99$, $p = 0.323$	$z = -1.10$, $p = 0.270$	$z = -2.28$, $p = 0.023$

Number spp. clean	z = 3.16, p = 0.002	z = 6.65, p < 0.001	z = 9.69, p < 0.001	z = 7.36, p < 0.001	z = 8.75, p < 0.001	z = 7.66, p < 0.001	z = 13.56, p < 0.001	z = 12.63, p < 0.001
Number spp. local	z = -3.20, p = 0.001	z = -5.57, p < 0.001	z = -5.30, p < 0.001	z = -3.04, p = 0.002	z = -5.04, p < 0.001	z = -5.46, p < 0.001	z = -7.39, p < 0.001	z = -3.53, p < 0.001
Client local REL	z = -4.63, p < 0.001	z = -5.11, p < 0.001	z = -2.38, p = 0.017	z = -0.68, p = 0.498	z = 0.93, p = 0.354	z = -1.96, p = 0.050	z = -0.91, p = 0.362	z = 2.22, p = 0.027
Number spp. reef	-							
Abundance other cleaners	-							

(b) Cleaning duration

	2010	2011	2012	2013	2014	2015	2016	2017
Functional group	$\chi^2_3 = 11.97$, p = 0.007	$\chi^2_3 = 21.68$, p < 0.001	$\chi^2_3 = 3.44$, p = 0.328	$\chi^2_3 = 4.92$, p = 0.177	$\chi^2_3 = 11.55$, p = 0.009	$\chi^2_3 = 5.42$, p = 0.144	$\chi^2_3 = 1.85$, p = 0.605	$\chi^2_3 = 1.94$, p = 0.585
Client size	t = 0.90, p = 0.365	t = 0.85, p = 0.395	t = 4.57, p < 0.001	t = 2.17, p = 0.030	t = 0.58, p = 0.562	t = 1.47, p = 0.141	t = 1.83, p = 0.067	t = 0.56, p = 0.578
Client reef abundance	t = 0.40, p = 0.690	t = -4.25, p < 0.001	t = -2.40, p = 0.017	t = -1.44, p = 0.149	t = -2.18, p = 0.029	t = -0.98, p = 0.326	t = -0.30, p = 0.763	t = -0.68, p = 0.495

Number spp. reef	-

(c) Posing frequency

	2010	2011	2012	2013	2014	2015	2016	2017
Functional group	$\chi^2_3 = 5.62$, $p = 0.131$	$\chi^2_3 = 19.72$, $p < 0.001$	$\chi^2_3 = 15.61$, $p = 0.001$	$\chi^2_3 = 15.48$, $p = 0.001$	$\chi^2_3 = 128.13$, $p < 0.001$	$\chi^2_3 = 6.37$, $p = 0.095$	$\chi^2_3 = 35.02$, $p < 0.001$	$\chi^2_3 = 39.52$, $p < 0.001$
Client trophic	$z = -2.16$, $p = 0.031$	$z = -4.03$, $p < 0.001$	$z = -1.85$, $p = 0.065$	$z = -4.20$, $p < 0.001$	$z = -6.69$, $p < 0.001$	$z = -4.09$, $p < 0.001$	$z = -5.75$, $p < 0.001$	$z = -5.55$, $p < 0.001$
Client size	$z = -2.04$, $p = 0.041$	$z = -3.13$, $p = 0.002$	$z = -1.60$, $p = 0.111$	$z = 0.18$, $p = 0.857$	$z = 0.17$, $p = 0.863$	$z = -2.16$, $p = 0.031$	$z = -2.23$, $p = 0.026$	$z = 0.81$, $p = 0.418$
Client local abundance	$z = 1.51$, $p = 0.130$	$z = -1.89$, $p = 0.059$	$z = 0.75$, $p = 0.454$	$z = 0.73$, $p = 0.463$	$z = 1.96$, $p = 0.050$	$z = -2.77$, $p = 0.006$	$z = 4.54$, $p < 0.001$	$z = 5.54$, $p < 0.001$
Client reef abundance	$z = -1.39$, $p = 0.165$	$z = 3.48$, $p < 0.001$	$z = 3.59$, $p < 0.001$	$z = 0.74$, $p = 0.462$	$z = -0.66$, $p = 0.511$	$z = -0.25$, $p = 0.801$	$z = 3.79$, $p < 0.001$	$z = 1.51$, $p = 0.132$
Cleaner local abundance	$z = 0.22$, $p = 0.829$	$z = 0.10$, $p = 0.917$	$z = 0.29$, $p = 0.769$	$z = -1.25$, $p = 0.211$	$z = -1.04$, $p = 0.299$	$z = 0.18$, $p = 0.860$	$z = -2.96$, $p = 0.003$	$z = -1.63$, $p = 0.103$
Cleaner reef abundance	-							

Number spp. clean	$z = 0.56$, $p = 0.579$	$z = -0.63$, $p = 0.528$	$z = 2.48$, $p = 0.013$	$z = 2.66$, $p = 0.008$	$z = 2.74$, $p = 0.006$	$z = 0.64$, $p = 0.519$	$z = 3.37$, $p < 0.001$	$z = 2.73$, $p = 0.006$
Client local REL	$z = -2.19$, $p = 0.029$	$z = -2.38$, $p = 0.017$	$z = -2.65$, $p = 0.008$	$z = -3.11$, $p = 0.002$	$z = -6.33$, $p < 0.001$	$z = -2.19$, $p = 0.029$	$z = -1.37$, $p = 0.172$	$z = -3.54$, $p < 0.001$
Abundance other cleaners	-							

(d) Posing duration

	2010	2011	2012	2013	2014	2015	2016	2017
Functional group	$\chi^2_3 = 2.02$, $p = 0.364$	$\chi^2_3 = 18.23$, $p < 0.001$	$\chi^2_3 = 3.86$, $p = 0.277$	$\chi^2_3 = 11.93$, $p = 0.008$	$\chi^2_3 = 26.41$, $p < 0.001$	$\chi^2_3 = 3.80$, $p = 0.284$	$\chi^2_3 = 0.87$, $p = 0.832$	$\chi^2_3 = 6.10$, $p = 0.107$
Client trophic	$t = 0.22$, $p = 0.824$	$t = 1.36$, $p = 0.175$	$t = 2.76$, $p = 0.006$	$t = 1.05$, $p = 0.293$	$t = 1.51$, $p = 0.131$	$t = 1.54$, $p = 0.124$	$t = 0.25$, $p = 0.803$	$t = 0.09$, $p = 0.926$

Chapter 7

Cleaner Personality and Client Identity Have Joint Consequences on Cleaning Interaction Dynamics

A version of this chapter has been published in Behavioral Ecology:

Dunkley, K., Ioannou, C.C., Whittey, K.E., Cable, J. and Perkins, S.E. (2019). Cleaner personality and client identity have joint consequences on cleaning interaction dynamics. Behavioral Ecology. DOI: 10.1093/beheco/arz007

ABSTRACT

Mutualistic interactions involve two species beneficially cooperating, but it is not clear how these interactions are maintained. In many mutualisms, one species interacts with multiple species, and since partners differ in terms of the commodities they trade, partner identity will directly influence the decisions and behaviours of interacting individuals. This chapter investigated the consequences of within and between-species diversity on a model cleaner-client interaction in a natural environment, by quantifying the behaviour of both partners. The predominant Caribbean cleaner fish, the sharknose goby (*Elacatinus evelynae*), did show personality variation since repeatable individual differences in activity, boldness and exploratory behaviours were documented. Personality variation was associated with cleaner-client interactions: cleaner boldness and activity were significantly related to posing by clients and cleaning, respectively. Cleaner personality variation was also associated with the functional identity (sociality, mobility, body size and trophic level) of clients posing and being cleaned. This chapter thus demonstrates that partner identity can have consequences on mutualistic outcomes which will contribute to the context-dependency and highly heterogeneous patterns observed at a population level. Here it is also suggested that within and between-species differences have consequences on partner choice, a feature that has been previously thought to be absent from these cleaner-client interactions.

INTRODUCTION

Mutualistic interactions, where two species beneficially cooperate, are observed in all ecosystems (Bronstein 2015), yet it is still not clear how these interspecific interactions are maintained. Mutualisms often involve food resources (e.g. nectar and ectoparasites) being traded for a beneficial act (e.g. pollination, Landry 2012, parasite removal, Arnal et al. 2001), known as service-resource interactions (Holland et al. 2005), but not all partners are equal in terms of the commodities they trade (Palmer et al. 2015). These interspecific interactions involve two individuals directly interacting at any one time, and thus the behaviours and traits of one partner, could directly influence the behaviours and traits of the other (Wolf and Weissing 2012). Partner identity will hence underpin the behavioural responses and decisions of animals during these cooperative interactions, influencing when individuals interact, with whom, and by how much (McAuliffe and Thornton 2015). Currently, understanding of mutualisms is hypothesised to be context-dependent and highly heterogeneous (Bronstein 2015), so investigating how individual partners influence mutualism outcomes will help to clarify the dynamics and hence evolution of mutualisms under natural conditions.

Within an environment, service providers only make up a small proportion of the biomass but interact with a disproportionately large number of other species (Sazima et al. 2010). As a result, mutualisms are often composed of networks of interacting species, with service providers carrying out ecosystem services, such as pollination (Landry 2012) and health enhancing parasite control (Clague et al. 2011b; Waldie et al. 2011). Mutualists thus play a pivotal role in the structuring and functioning of ecological communities (Floeter et al. 2007; Sazima et al. 2010; Quimbayo et al. 2018). An iconic, well studied service-resource mutualism, the cleaner-client interaction, is observed ubiquitously on coral reefs (White et al. 2007; Leung and Poulin 2008). The mutualism involves a cleaner removing ectoparasites and other material from the bodies of many client fish species (up to 132 different species, Grutter and Poulin 1998b). Cleaning patterns, however, are inconsistent, with the same cleaner species showing preferences for different client types across studies. For example, cleaning gobies from the genus *Elacatinus* prefer larger clients in some studies (e.g. Whiteman and Côté 2002a; Silvano et al. 2012), but not in others (e.g. Arnal et al. 2000). These, like many other behavioural studies, focus on population patterns, which assume all conspecifics exhibit the same traits, or that variation around an average is random (Bolnick et al. 2011). Individuals within many invertebrate and vertebrate populations vary consistently in their behaviour (also known as animal personality variation, Réale et al. 2007), and this variation can play a major role in shaping population-level patterns of species interactions and other ecological processes (Wolf and Weissing 2012). There are five recognised animal personality traits (Réale et al. 2007), and for many taxonomically distinct species, these traits can affect feeding and foraging behaviours. The personality traits boldness and exploration, for example, which can be broadly defined as an individual's reaction to a risky (boldness) and new situation (exploration) (Réale et al. 2007), influence both an individual's food intake and foraging success (Ioannou et al. 2008; David et al. 2011). Bolder and more exploratory individuals are expected to have increased metabolic demands since they are at an increased risk (e.g. to predation) and utilise the environment more widely (Careau et al. 2008; Brommer and Class 2017). A third personality trait, activity, which quantifies the general activity level of an individual (Réale et al. 2007), may also often predict foraging behaviours (Pruitt et al. 2012) as more active individuals are also expected to have increased energy demands (Careau et al. 2008; Brommer and Class 2017). Thus, personality traits, and their correlations with one another (forming a behavioural syndrome; Sih et al. 2012) are likely to play a role in food acquisition during mutualistic interactions: dedicated cleaners for example, gain all their nutrition from client derived material (Vaughan et al. 2017). Indeed, bolder cleaner fish (*Labroides dimidiatus*) have been shown to clean less honestly (i.e. cheat more) to acquire a more favourable reward (Wilson et al., 2014), whilst bolder black-billed magpie cleaner birds (*Pica pica*) interact with clients more frequently, facilitating greater access to protein-rich ticks (Found 2017).

The dynamics of mutualistic interactions are not just driven by a cleaner's food dependency however (Lenke 1988), since the resource provider's behaviour, engagement and traits can also regulate outcomes of an interaction (Bever 2002; Bshary and Schäffer 2002). In cleaning interactions, clients can choose which cleaners to visit (Bshary and Schäffer 2002), and increase their chances of being cleaned (Côté et al. 1998), by presenting their body to cleaners (termed posing, Feder 1966). However, posing does not necessarily guarantee cleaning, and for some clients, they need not pose at all to be cleaned (Arnal et al. 2001). The cleaners past behaviour towards the client can also influence their interactions with different cleaners: if a client has received a negative response from the cleaner, for example, they are less likely to revisit (Bshary and Schäffer 2002). Cleaners thus adapt their behaviours to ensure client satisfaction (Grutter and Bshary 2003). Partner feedbacks are hence an important component for maintaining positive inter-specific interactions (Frederickson 2013), yet their role is largely ignored. Given that feedbacks can reinforce the development of behaviours (Houston and McNamara 1999; Sih et al. 2015), it would be expected that the expression of personality variation by cleaners would link with both the actor's and receiver's behaviour. This prediction however has not yet been tested in a cleaning context, but personality variations have been shown to mediate other interaction types (e.g. predator-prey interactions, Pruitt et al. 2012, and service-service mutualisms, Schmiege et al. 2017). Client species differ in their propensity to engage in cleaning interactions (Côté et al. 1998; Bshary and Schäffer 2002), as well as the nutritional content that they represent to cleaners (Eckes et al. 2015). These differences mean that different clients will provide asymmetric benefits to the cleaning interaction. Larger (Poulin and Rohde 1997), group living and sedentary (Patterson and Ruckstuhl 2013) species, for example, are more prone to increased parasite loads. It is unknown whether individual cleaners respond asymmetrically to client identities and vice versa, influencing interaction patterns.

Here, to investigate the consequences of within and between-species diversity on the outcome of mutualistic interactions, both cleaner and client behaviour *in situ* was quantified. The cleaning interactions between the predominant Caribbean cleaner fish, the sharknose goby (*Elacatinus evelynae*), and their reef fish clients were observed. These cleaner species rarely cheat by causing damage to client bodies (Soares et al. 2008b), and thus their cleaning behaviour represents a simpler system for studying cleaner-client interactions compared to the iconic bluestreak wrasse cleaners (*Labroides dimidiatus*, Côté and Soares 2011). Previous work has documented personality variation in (non-cleaning) goby species (e.g. Magnhagen et al. 2014; Moran et al. 2016; Vallon et al. 2016), and as such, it was expected that sharknose gobies show individual variation in major axes of personality traits (activity, boldness and exploration). As personality traits can influence foraging behaviours, and clients will differ in the food material they host, it was then determined whether different personality variations had consequences on cleaning behaviours (frequency, rate and which clients cleaners interacted with). Finally, since clients can also regulate mutualistic outcome patterns, this chapter tested whether clients interacted differently with cleaners based on the cleaners' personality traits (posing frequency, rates and client functional identity).

METHODS

Identifying individual cleaner fish

Sharknose goby (*Elacatinus evelynae*) behaviour was observed on Booby Reef, Man O' War Bay, Tobago (11°19.344′N 060°33.484′W) over a two week period in June to July 2017 by daily snorkeling between the hours of 07:00 and 17:30. This study took place in the last two weeks of a six week field season, and thus gobies were assumed to be habituated to human presence on the reef. The small section of the reef sampled (60 m x 70 m; reef depth: 1 – 2 m) is composed predominantly of algae covered dead elkhorn coral (*Acropora palmata*) and living brain coral (*Diploria* spp.). Individual sharknose gobies show strong site fidelity to their brain coral cleaning stations (Whiteman and Côté 2002b; Harding et al. 2003), so individuals (n = 17) could be repeatedly identified based on their cleaning station. The cleaning stations used in this study have been monitored annually as part of a long-term study (8 year, 2010 – 2017) and are located at least 1 m apart from one another. Cleaning interactions do not differ spatially on the reef (**Chapter 6**). Sharknose gobies have a high turnover rate on their cleaning stations (mean age < 50 days documented in White et al. 2007), so it was not possible to quantify personality variation of the same individuals across years. Where more than one goby occupied a station (up to 4 gobies), a focal was identified based on relative size differences and only one individual was chosen per station.

Quantifying cleaner fish personality variation

Cleaner fish personality variation was quantified *in situ* at their fixed cleaning stations. Activity was determined through un-manipulated observations, whilst boldness and exploration were quantified using stimuli. To prevent habituation to the stimuli presented, boldness and exploration were quantified twice per stimulus (Figure 7.1). This study did not test for individual variations in the aggressiveness and sociability axes of personality, since cleaning gobies are often found singularly or in small groups (Whiteman and Côté 2002b). All behaviours were recorded by observers and were not filmed due to the heterogenous nature of the environment; cleaners often traverse around their large coral heads (ca. 1 – 2 m^3) and thus could be regularly out of view from fixed cameras. Given that environmental variables, and the timescale between observations, can influence the consistency of behaviours (Bell et al. 2009; Wong et al. 2017; Pruitt et al. 2018), and here personality variations were quantified in the field, assays were repeated on consecutive days (where possible). The order of presenting the stimuli in the boldness and exploration assays was randomised across individuals; individuals experienced only one stimulus per day, and this occurred at a random time point. For all observations (n = 173), observers maintained a distance of 1.5 m from the cleaner.

I. *Activity*

Activity (for n = 17 individuals) was quantified over a 10 minute observation prior to a stimulus being presented in the boldness and exploration assays so that stimuli presentation did not interfere with quantifying activity (Figure 7.1). In contrast to the mid-water wrasse cleaners (e.g. *Labroides dimidiatus*), coral-dwelling sharknose gobies remain in direct contact with the coral at their cleaning stations (apart from when cleaning, and the occasional competition-induced move to adjacent coral, Whiteman and Côté 2002b; Côté and Soares 2011). Thus, within each observation, activity was measured as: the total 2D distance travelled by the cleaner across the coral surface or when swimming in open water, estimated to the nearest 5 cm (or to the nearest 1 cm if distance travelled < 5 cm), the total duration of these movements, and the total duration of 'jerk' movements (localised movement where the cleaner does not cover any distance over the coral head). To investigate behavioural consistency (Réale et al. 2007), activity was recorded up to six times (n = 6 for 12 individuals, n = 5 for one individual and n = 2 for four individuals, dependent upon whether individuals were seen on their station, Figure 7.1).

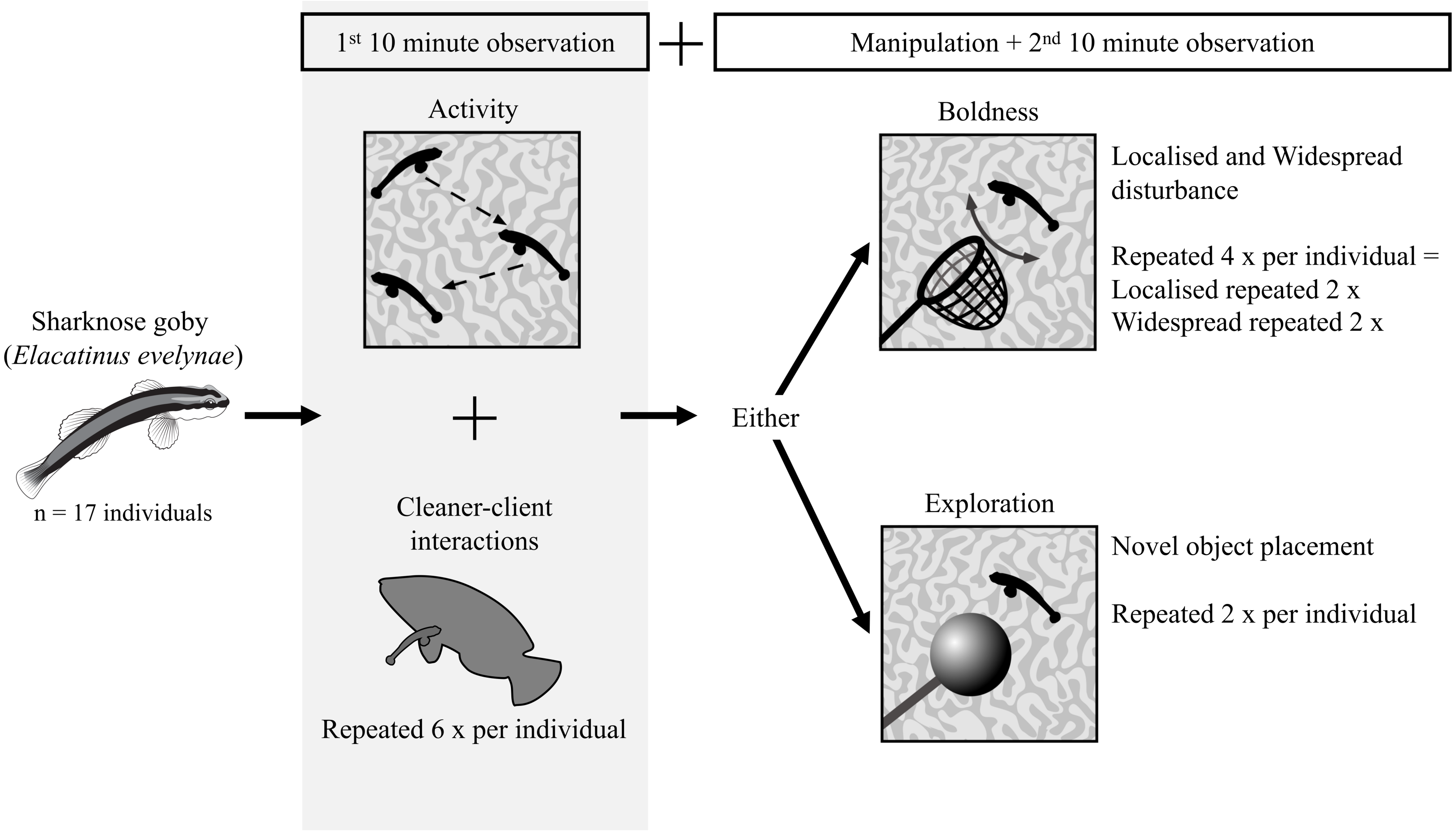


Figure 7.1: Methods for quantifying sharknose goby (*Elacatinus evelynae*) personality variation (activity, boldness and exploration) *in situ*. Individual gobies were identified from their cleaning stations, and personality assays (boldness versus exploration) were conducted on separate days using different stimuli. Activity quantification was carried out before each stimuli presentation. Recorded behaviours are listed in text and in Table 7.1.

II. Boldness

The shyness-boldness axis of personality variation represents an individual's reaction to a risky situation (Réale et al. 2007), so each focal cleaner (n = 15) was disturbed using both a localised and widespread disturbance for 20 seconds. Boldness behaviour was based on four stimuli presentations; each cleaner was disturbed twice by both disturbance methods (localised versus widespread, Figure 7.1, n = 14 individuals disturbed by both methods, n = 1 individual only disturbed by a localised disturbance). The local disturbance involved proximally and distally moving a bamboo cane 10 cm from the focal cleaner, whilst a net (10 cm x 10 cm) was moved 1 m laterally to the coral head to create a widespread disturbance. The local method hence created a disturbance at the cleaner's position on the station (representing a single client disturbing the cleaner), whilst the widespread method created a larger disturbance over the cleaner's position and surrounding coral head (representing a shoaling client group disturbance). Typically, the focal cleaner quickly moved away from its position on the coral head during both disturbances. Following disturbance, the fish was observed for 10 minutes and the time taken for the individual to return to the original pre-disturbance location was recorded. Within this 10 minute observation the cleaner's activity behaviour was also recorded (as described previously). This protocol meant that an individual's pre- versus post-disturbance movement behaviour could be directly compared to quantify how an individual initially responded to a risky situation (similar to Houslay et al. 2018).

III. Exploration

Exploration represents an individual's reaction to a new situation (Réale et al. 2007), so cleaners (n = 16) were presented twice with a novel object (sand-filled orange ping-pong ball attached to a green garden cane weighted in a sand-filled bottle). The ball was placed 10 cm away from, but at the height of, the cleaner's position on the station for 10 minutes and exploration was measured as the time taken for the cleaner to approach within 20 cm of the ball, the cleaner's closest distance to the ball (to the nearest 1 cm if < 5 cm and to the nearest 5 cm if > 5 cm away), and the time taken for the cleaner to return to its original location. Cleaners were observed inspecting and moving on the novel object. Observers placed the object as quickly and smoothly as possible to minimise and standardise any additional disturbance.

Quantifying cleaner-client interactions

During the 10 minute observations quantifying a cleaner's activity behaviour, any cleaner-client interactions were also recorded (Figure 7.1). Cleaning behaviours only took up a small proportion of the observation (mean ± S.E. = 10.6 seconds ± 2.09). Recorded behaviours were: the duration and frequency of cleaning of, and posing by, client species during each observation. Posing and cleaning rates, and frequencies within the observation, were used as a measure of client-cleaner behaviour. The frequencies represent the total effort in cleaning or posing across all client species, while the rates are this effort per cleaning time (i.e. total cleaning frequency / total cleaning duration). Cleaners were not always in view to the observer due to the heterogeneous nature of their cleaning stations, thus the time a cleaner was out of view within each observation was accounted for by dividing frequency and duration data by the adjusted observation lengths.

Data analysis

Data were analysed using R, version 3.4.3 (R Core Team 2017). Generalised Linear Mixed Models (GLMMs) were run using the lme4 package (Bates et al. 2015). For all models, response variables were rescaled from one to 10 using the scales package (Wickham 2017). This rescaling method does not remove the variability in responses, but simply transforms the small and/or negative variables to aid model fit. Cook's distance identified influential points and models were re-run without them to determine their effect; influential points are only reported if they had a significant influence on the results. Model assumptions and fits, as specified by Bolker et al. (2009) were assessed using residual plots and all continuous predictors were scaled and centred around zero to facilitate model convergence.

The behaviours measured during the activity trials were included in a Principal Component Analysis (PCA) to produce an activity measure for each observation for each individual (n = 87 observations, n = 17 individuals); the same method was used to produce boldness (n = 56 observations) and exploration scores (n = 30 observations, behaviours and PC1 loadings listed in Table 7.1).

Table 7.1: PCA loadings of behavioural variables used to generate first principal component scores (PC1) to quantity individual sharknose goby (*Elacatinus evelynae*) activity, boldness, and exploration scores.

Personality trait	Recorded behaviours	PC1 loadings	Variation explained
Activity	Proportion of observation spent moving	0.508	47.22%
	Distance moved within observation	0.554	
	Speed	0.281	
	Frequency of movements that covered distance	0.564	
	Frequency of jerk movements	0.189	
	Frequency of open swims	0.012	
Boldness	Return time after disturbance by stick or net	-0.048	41.68%
	Difference in proportion spent moving pre- vs post-disturbance	-0.589	
	Difference in distance moved pre- vs post-disturbance	-0.546	
	Difference in speed pre- vs post-disturbance	0.098	
	Difference in jerk frequency pre- vs post-disturbance	-0.570	
Exploration	Time taken to return to position following novel object placement	-0.698	60.84%
	Time taken to be < 20 cm from object	-0.701	
	Closest distance to novel object	-0.145	

Thus, multiple scores were calculated for each individual (max n = 6 scores for each individual for each personality trait). The first principal component score of each PCA was used as the measure of an individual's boldness, activity and exploration in each observation (as in Wilson et al., 2014). Table 7.1 shows the loading of each behavioural measure on each PC1 score. Prior to score calculation, behaviour values were standardised using mean centring, and thus both negative and positive PC1 scores occurred.

To determine whether cleaners show personality variation in activity, boldness and exploration, data were analysed at an observation (i.e. per trial) level. GLMMs were used to analyse sharknose goby activity (Gamma family with inverse link function), exploration (inverse Gaussian family with inverse link) and boldness (inverse Gaussian family with inverse link, boldness scores were reverse transformed) scores from the PCAs. The following main effects were included within each behavioural trait analysis: day from the start of the study, time of day, time since last observation, number of cleaners at the station, disturbance order (based on presented order of three disturbances: cane, net and novel object), replicate of each disturbance (for boldness and exploration: one or two) and observer ID. For boldness, the disturbance method (cane versus net) was also included as a main effect. Models were refined by removing the least significant term in each step. For all three GLMMs (activity, boldness and exploration as the response variables), individual identity was included as a random term. For the best fitting models, likelihood ratio tests (LRTs) comparing models with and without this random effect of individual (similar to Houslay et al. 2018) determined whether among-individual differences existed in activity, boldness and exploration scores.

To investigate the role of behavioural traits on cleaner-client interactions, an individual's mean PC1 score was calculated separately for activity, boldness and exploration behaviours. Pairwise correlations are widely used to investigate behavioural syndromes (Sih et al. 2012), and thus Spearman rank correlation tests determined whether individual sharknose gobies mean activity, boldness, and exploration scores related to one another in a behavioural syndrome. Individual sharknose goby mean activity, boldness and exploration scores lie at different locations along continua, and thus for further analysis, this between individual variation was not excluded. As such, simple GLMs were used for all further analyses rather than non-parametric Spearman rank tests. The significance of main effects was determined by comparing models with and without the main effect.

To provide a measure of each cleaner individual's cleaning interactions, an individual's mean frequency and rate of cleaning and posing were also calculated from activity observations (i.e. before disturbances). Shannon's diversity indices were calculated across all observations for each individual using the vegan R package (Oksanen et al. 2018) to provide a measure of the client diversity being cleaned and posing for each focal cleaner. Due to the small sample size (max = 17 individuals) and numerous predictors, forward stepwise GLMs determined whether an individual's mean activity, boldness and exploration behaviours predicted their mean cleaning frequency and rate (both Gamma family, log link), and diversity of clients cleaned

(Gaussian family, log link). Predictors were initially kept in the model based on a critical $\alpha = 0.157$ (Heinze et al. 2018), whilst the final model only contained significant predictors. The same method was used for mean posing frequency (Gamma family, log link), rate (inverse Gaussian family, log link) and diversity (Gamma family, log link). The main effects of mean activity, boldness and exploration, observer ID and mean number of cleaners on the station were sequentially and manually added to the model based on descending correlation coefficients between each variable and the response variable. The main effects of cleaning frequency, rate and diversity cleaned, and posing frequency, rate and diversity posed were also added, where they were not considered as the response variable, to control for any feedbacks in behaviour, since solicitation behaviours can initiate cleaning interactions.

To determine whether cleaner personality variation is associated with which clients are involved in and engage with cleaning interactions, client species were assigned maximum fork lengths using (Humann and Deloach 2014) and trophic levels using FishBase (Froese and Pauly 2018). A trophic level of two indicates an herbivorous client, whilst levels of three and above represent predatory clients. FishBase was also used to record clients as either solitary or gregarious (associate with > 3 individuals) and sedentary or free-ranging. Including all the clients a sharknose goby cleaned, and their mean activity, boldness and exploration scores, three GLM's determined whether sharknose goby behavioural traits predicted which clients were cleaned in terms of their size (inverse Gaussian family, inverse link), trophic level (two versus three, binomial family, probit link), sociality (gregarious versus solitary, binomial family, logit link) and mobility (free-ranging versus sedentary, binomial family, probit link). A further four GLM's determined whether posing client traits were predicted by activity, boldness and exploration scores (size: inverse Gaussian family, log link, trophic level: binomial family, logit link, sociality: binomial family, cauchit link, mobility: binomial family, probit link).

RESULTS

Do cleaners show personality variation?

Individual sharknose gobies differed from one another in their activity (LRT, $\chi^2_1 = 5.21$, $p = 0.022$, final model ${}_{adj}R^2 = 14.2\%$), boldness (LRT, $\chi^2_1 = 8.78$, $p = 0.003$, ${}_{adj}R^2 = 29.9\%$), and exploratory (LRT, $\chi^2_1 = 6.28$, $p = 0.012$, ${}_{adj}R^2 = 28.4\%$) behaviours, showing inter-individual variation in these three traits (S.E. minimum – maximum across individuals: activity = 0.21 – 1.10, boldness = 0.07 – 1.98, exploration = 0.15 – 2.13, Figure 7.2). An individual's mean boldness, activity and exploration scores, however, did not significantly correlate with one another to form a consistent behavioural syndrome (between trait correlations: activity – bold Spearman's rho = -0.279, activity – exploration rho = 0.036, boldness – exploration rho = -0.071, all $p > 0.10$).

Although cleaners showed repeatable activity, boldness and exploration behaviours, these traits were also affected by external factors. Cleaners were more exploratory as the time into day increased (GLMM, $\beta = 0.06$, $\chi^2_1 = 4.05$, $p = 0.044$) and there was evidence for habituation as activity scores increased over the sampling period (GLMM, $\beta = 0.03$, $\chi^2_1 = 4.93$, $p = 0.026$) and cleaners were bolder on the second replicate of each stimulus presentation compared to the first, irrespective of method (GLMM, $\beta = 0.06$, $\chi^2_1 = 5.84$, $p = 0.016$), although boldness did decrease across the sampling period (GLMM, $\beta = -0.10$, $\chi^2_1 = 9.53$, $p = 0.002$). Previous studies have documented social context influencing the expression of personality traits (Webster and Ward 2011; McDonald et al. 2016; Bevan et al. 2018), but here the number of cleaners occupying a station did not affect personality scores (GLMMs, $p > 0.05$).

Is personality variation associated with cleaner-client interactions?

The three personality axes of activity, boldness and exploration, linked to cleaner-client interactions. More active gobies cleaned a lower diversity of clients and cleaned at a lower rate, whilst bolder individuals experienced an increased posing frequency by their clients. Exploration had no effect on cleaner-client interactions (Figure 7.3).

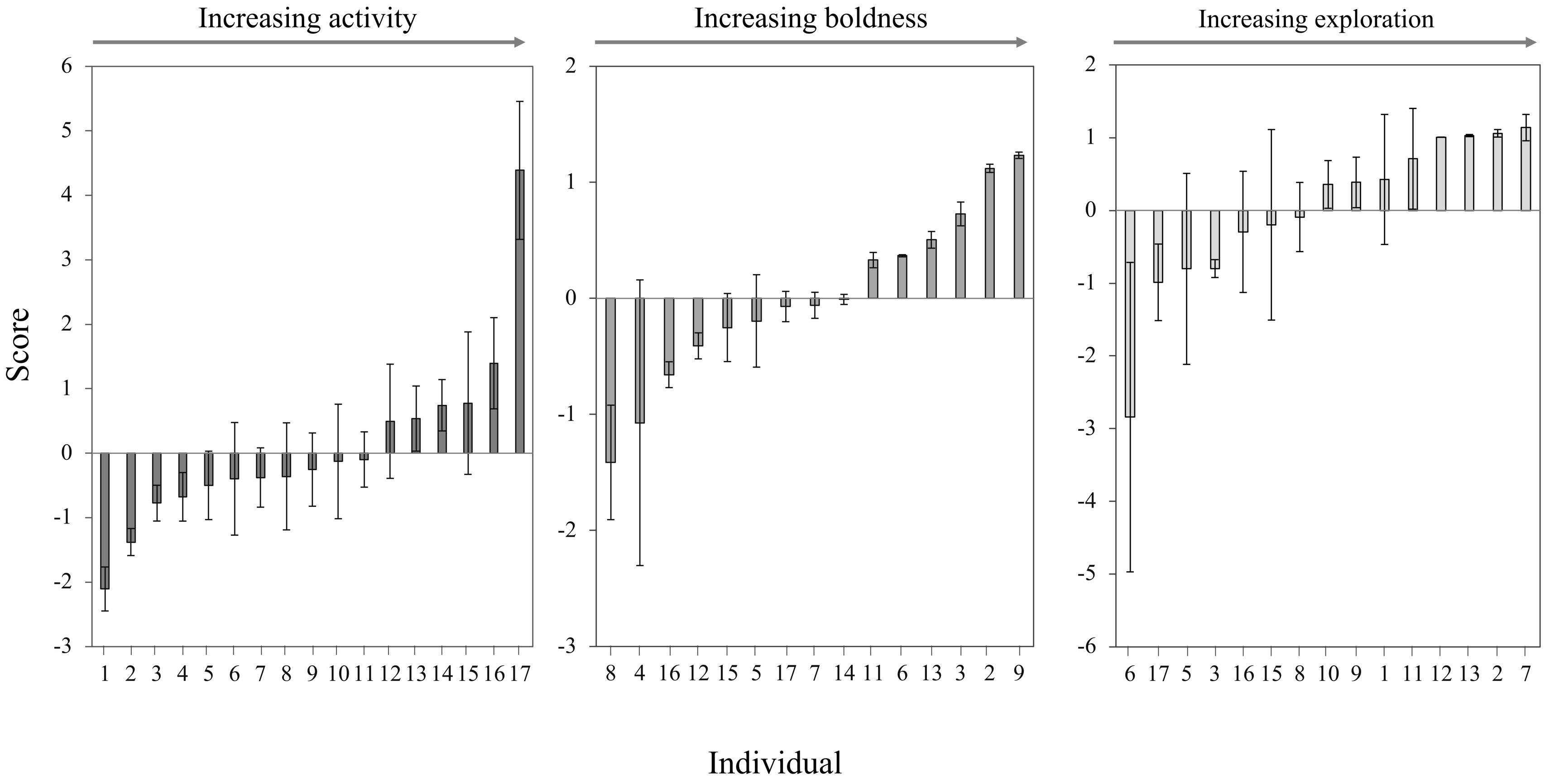


Figure 7.2: Mean (± S.E.) activity, boldness and exploration scores (PC1; Table 7.1) for individual sharknose gobies (*Elacatinus evelynae*) occupying cleaning stations on Booby Reef Man O’ War Bay, Tobago. Individual activity scores are based on a maximum of six replicates, whilst boldness scores are calculated from two disturbance methods (cane and net) both repeated once, and exploration was quantified twice per individual.

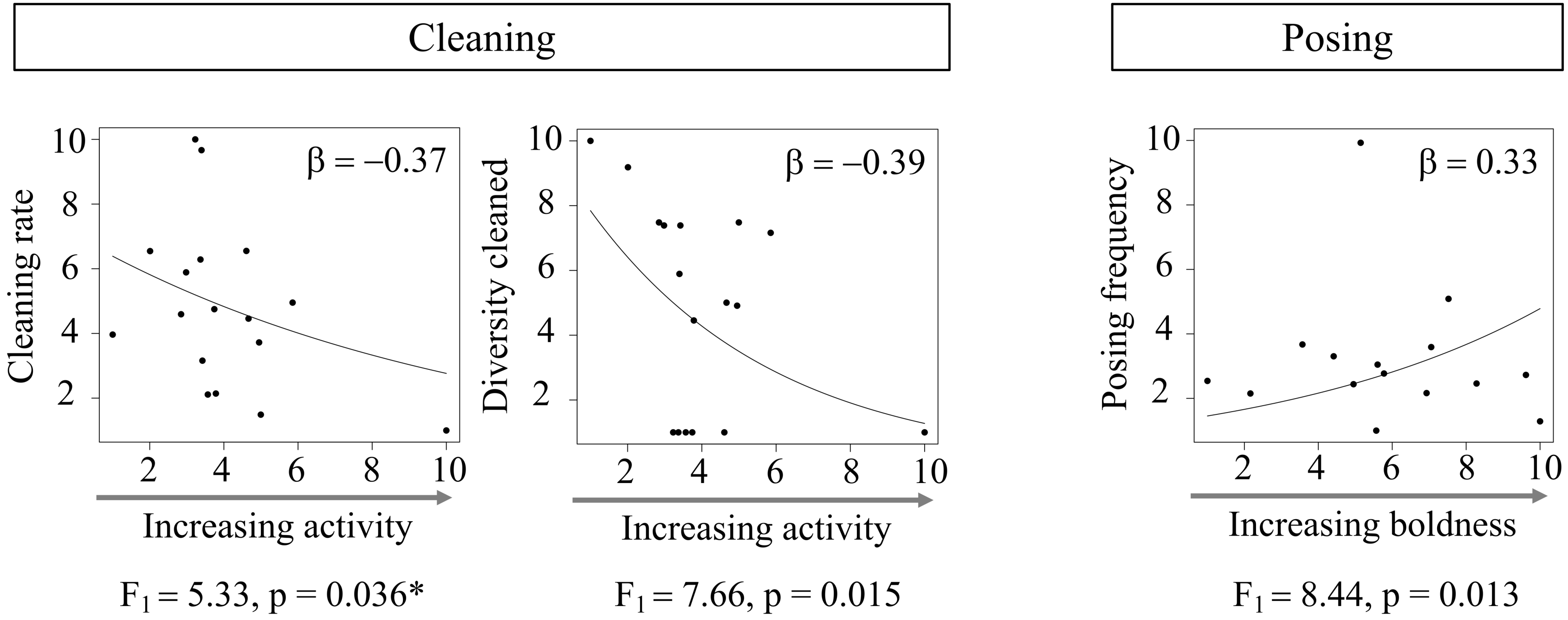


Figure 7.3: Relationships between sharknose goby (*Elacatinus evelynae*) personality scores and cleaning and client behaviour. Significant GLM relationships between activity score and mean cleaning rate and diversity of clients cleaned, and boldness score and mean experienced posing frequency (all rescaled), with lines based on model coefficients. * p = 0.440 without influential point. Exploration scores did not predict cleaner-client interactions.

Reciprocal positive feedbacks between individual cleaners' cleaning frequencies and client posing frequencies across cleaners were also found (GLMs: clean frequency – pose frequency: $\beta = 0.41$, $p < 0.001$, final model $_{adj}R^2 = 46.3\%$; pose frequency – clean frequency: $\beta = 0.57$, $p < 0.001$, $_{adj}R^2 = 68.8\%$). Client posing rates were also positively predicted by cleaners cleaning rates (GLM, $\beta = 0.49$, $p = 0.002$ $_{adj}R^2 = 59.3\%$) and negatively related to cleaning frequencies (GLM, $\beta = -0.48$, $p = 0.001$). The diversity of posing clients also correlated positively with the diversity of clients cleaned and vice versa (GLM, diversity cleaned – diversity posed $\beta = 0.41$, $p = 0.001$, $_{adj}R^2 = 64.8\%$, diversity posed – diversity cleaned $\beta = 0.62$, $p < 0.001$, $_{adj}R^2 = 66.7\%$). Contrary to expectation, given the generally positive relationships between cleaner and client behaviour, bolder individuals, which experienced an increased posing frequency did not clean more, and more active individuals which cleaned less frequently, did not experience more posing behaviour (frequency and rate) from clients.

Is cleaner personality variation associated with client traits?

Across this study, sharknose gobies cleaned 16 client species across 96 cleaning events, and cleaner personality variation was associated with which clients were cleaned. Bolder individuals cleaned herbivorous clients, whilst shyer gobies cleaned higher trophic level clients (Figure 7.4, GLM, $\chi^2_1 = 8.14$, $p = 0.004$, final model $_{adj}R^2 = 46.1\%$). Albeit low $_{adj}R^2$ values, individuals considered most exploratory cleaned larger clients (Figure 7.4, GLM, $F_1 = 4.67$, $p = 0.033$, $_{adj}R^2 = 4.9\%$) and the free-ranging fish (Figure 7.4, GLM, $\chi^2_1 = 7.27$, $p = 0.007$, $_{adj}R^2 = 8.3\%$). Cleaner activity did not influence which clients were cleaned (GLM, sociality, mobility, trophic level and size all $p > 0.10$).

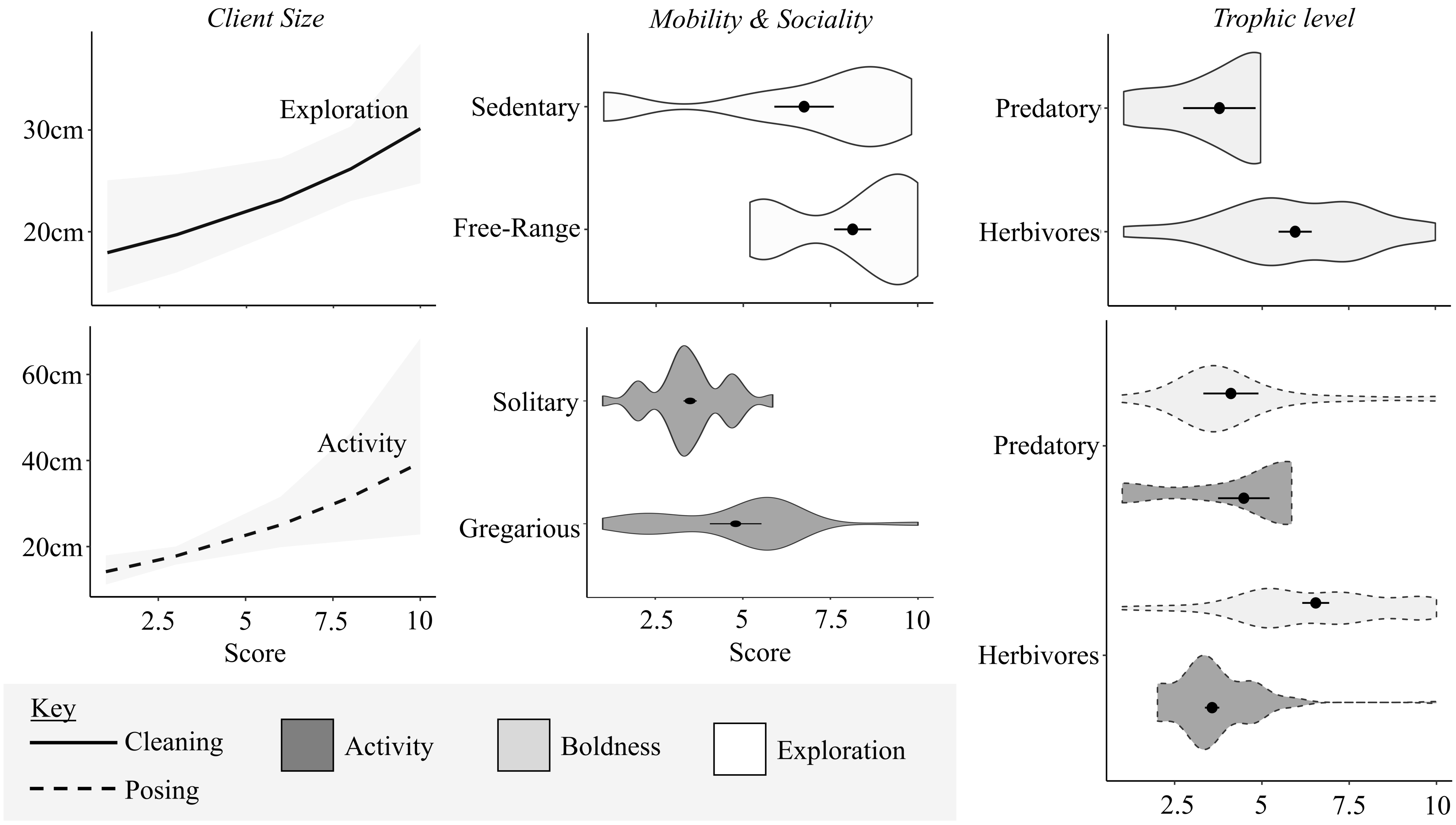

Client Size
Mobility & Sociality
Trophic level
Exploration
30cm
20cm
Sedentary
Free-Range
Predatory
Herbivores
Activity
60cm
40cm
20cm
Solitary
Gregarious
Predatory
Herbivores
2.5
5
7.5
10
Score
2.5
5
7.5
10
Score
2.5
5
7.5
10
Key
Cleaning
Posing
Activity
Boldness
Exploration

Figure 7.4: Relationship between sharknose goby (*Elacatinus evelynae*) personality scores and the identities of the clients cleaned and posing. Significant associations from GLMs between activity, boldness and exploration scores, and the clients cleaned (solid line) and posed (dashed line). PC1 scores (shown in Figure 7.1) were rescaled from one to 10 and mean scores were used in GLMs for each individual cleaner. Clients species are defined in terms of their functional traits: sociality, mobility, trophic level and body length (cm). Herbivores are defined as having a trophic level from 2 – 2.9, whilst predators represent the clients which have trophic levels > 3. Line figures represents effects of mean activity, and exploration scores (from GLMs) across the range of client body sizes (minimum = 9 cm, maximum = 60 cm) observed posing and/or cleaned. Shaded regions show 95% CI. The outer shapes on the violin plot represent the range of mean personality variation scores over which different client types (sociality, mobility and trophic level) posed to and were cleaned by, different sharknose gobies. The thickness of each shape represents how frequently these client types posed to (dashed line) and were cleaned by cleaners with different activity, boldness and exploration mean scores. Point and lines show mean ± 95% CI.

Seventeen client species posed for cleaners across 143 posing events with a total of 22 different species being involved in cleaner-client interactions. From a client's perspective, larger fish posed for more active individuals (Figure 7.4, GLM, $F_1 = 13.03$, $p < 0.001$, ${}_{adj}R^2 = 8.2\%$), as did the more predatory species (Figure 7.4, GLM, $\chi^2_1 = 18.19$, $p < 0.001$, ${}_{adj}R^2 = 43.8\%$). The more predatory clients also posed for the shyer cleaners (Figure 7.4, GLM, $\chi^2_1 = 8.04$, $p = 0.005$, ${}_{adj}R^2 = 43.8\%$). Finally, solitary fish posed for less active cleaners over more active cleaners (Figure 7.4, GLM, $\chi^2_1 = 35.32$, $p < 0.001$, ${}_{adj}R^2 = 25.2\%$). Cleaner exploration did not associate with which clients posed to cleaners (GLM, sociality, mobility, trophic level and size all $p > 0.10$).

DISCUSSION

This field study demonstrates that sharknose goby (*Elacatinus evelynae*) cleaners show personality variation with consistent inter-individual variation in their activity, boldness and exploration behaviours. Both activity and boldness were linked with cleaner-client interactions: more active cleaners cleaned a lower diversity of clients at a lower rate, whilst bolder individuals experienced an increased posing frequency by their clients. Personality variation was associated with client functional traits (sociality, mobility, trophic level and body size), influencing which client species interacted with an individual goby of a given personality type. In summary, this chapter shows that within and between-species diversity has consequences on mutualistic outcomes.

Personality variation in activity influenced goby cleaner-client interaction dynamics. Due to increased metabolic demands, more active individuals are expected to increase their foraging behaviour (Careau et al. 2008; Brommer and Class 2017), but here, more active individuals cleaned at a lower rate, and cleaned a lower diversity of clients. For other cleaner species, active behaviours (e.g. dancing, Youngbluth 1968, clapping, Chapuis and Bshary 2010, and rocking, Becker and Grutter 2005) attracts clients, but here the most active cleaners were not visited more frequently by client fish, suggesting gobies do not use obvious advertising movements. Given that sharknose goby cleaners gain all their nutrition from client derived material (Vaughan et al. 2017), more active gobies are utilising a more limited resource (reduced cleaning rate and diversity of clients cleaned) for foraging gains. Therefore, they could be more efficient cleaners, or else the trait would not be expected to persist. An increased cleaning efficiency may explain why larger fish posed for more active gobies. Larger bodied fish tend to host more parasites (Poulin and Rohde 1997), and will also gain a greater cost when posing: posing temporarily stops a client from foraging (Grutter et al. 2002) and larger fish have increased energy demands (Bachiller and Irigoien 2012). Clients can learn the identity of specific cleaners from past positive experiences (Bshary and Schäffer 2002) or from observing how other individuals have been treated by the cleaner (Bshary 2002b), thus visiting more efficient cleaners could reduce a client's costs associated with cleaning. Conversely, more active gobies may not need to be efficient since here they interacted with all client types: more active gobies would thus not be restricted in the types of food resources available. A future study comparing the diets (in terms of nutritional gains) between cleaner gobies with contrasting levels of activity would be useful for determining how important these traits are for goby fitness in a foraging context.

Boldness influences foraging behaviours across many species (Réale et al. 2007; Biro and Stamps 2008; David et al. 2011), but here bolder cleaners did not differ in their cleaning behaviour (i.e. foraging rates/frequencies) compared to shyer fish (contrasting Wilson et al. 2014). Partner choice can facilitate cooperation (Noë 2001), and bolder individuals were visited more frequently by clients compared to shyer individuals. Bolder animals are greater risk takers by definition (Réale et al. 2007); bolder *Labroides dimidiatus* cleaners for example, take risks by cheating their clients more frequently than shyer fish (Wilson et al. 2014). Although in other interaction contexts, bolder individuals are more likely to initiate and lead conspecific interactions (Ioannou and Dall 2016), a beneficial trait for posing clients, bolder individuals may risk not interacting with, and appeasing, all clients. Instead, bolder fish may reduce their own energetic costs by only cleaning preferred clients for maximum benefit (facilitated by an increased abundance of client fish posing for them creating choice options). Indeed, bolder individuals only cleaned herbivorous clients which feed intensely on the benthos throughout the day (Hay 1997). Benthic feeding brings potential clients in direct contact with the mobile crustacean ectoparasites which are often consumed during cleaning (Arnal et al. 2001; Grutter 2002), thus these clients may host high parasite loads and hence food rewards.

Exploration tendency increases how efficiently individuals utilise environments (Careau et al. 2008; Brommer and Class 2017), and although exploration did not link with cleaning behaviour (contrasting Wilson et al. 2014), more exploratory cleaners differed in which clients they cleaned (more exploratory individuals cleaned larger clients and the free-ranging fish). Larger clients are assumed to be prone to increased parasite loads (Poulin and Rohde 1997) and being more exploratory may enable cleaners to quickly find parasites over a larger surface area: exploration is a measure of speed with which an individual moves around a novel environment (Réale et al. 2007). In contrast, free-ranging clients are assumed to host fewer parasites compared to sedentary species (Patterson and Ruckstuhl 2013), and thus being more exploratory may also facilitate cleaners to find and exploit more patchily distributed food sources (Mathot et al. 2012).

Mutualisms are maintained by positive interactions between partners, and for clients interacting with a cleaner they pay a cost. Thus clients must be responded to beneficially for them to return (Bshary and Schäffer 2002). Although strong feedbacks between posing and cleaning behaviour were documented, this was not reflected at an individual level. Cleaning behaviours expressed towards clients by more active, more exploratory or bolder fish did not reflect client posing behaviour and vice versa. The identities of clients cleaned versus those posed also did not align, with the exception of herbivorous fish posing to and being cleaned by bolder gobies. Cleaner gobies are thought to rarely cheat by causing damage to client bodies (Soares et al. 2008b), but this selective strategy for certain clients, irrespective of who is posing, may represent a subtler form of dishonesty. Overall, through partner identity, choice and behaviour, sharknose gobies with certain personality variations may reduce the maintenance of the mutualism in terms of the positive feedback between cleaning and posing.

Mutualisms involve many different asymmetric partners interacting with one another, and for the iconic cleaner-client interaction, within and between-species diversity can influence mutualism outcomes. This chapter demonstrate that there are asymmetries in interaction outcomes between different individuals, which will create heterogenous patterns at the population level, a common feature across studies of cleaner-client interactions. Here, within-species individual differences (of cleaners) linked with between-species differences (of clients), contributing to who interacts with whom. Sharknose goby cleaning interactions have often been regarded as simple cleaning interactions with cleaner and client behaviours having no consequences on the interacting partner (Soares et al. 2008b; Côté and Soares 2011). However, through behavioural feedbacks, and the expression of differing traits, partner behaviours and identities can strongly influence mutualisms, albeit in a subtler way than those observed for the bluestreak wrasse (*Labroides dimidiatus*). Ultimately, this work may be applied to aquaculture, where cleaner fish are currently inefficiently deployed to biologically control ectoparasites of farmed fish (see Rae 2002). Given that client identity is fixed in these systems, this study suggests that selecting cleaners based on their behavioural traits (as suggested by Powell et al. 2018) or altering personality types through training (e.g. Frost et al. 2007) may increase the efficiency of deployed cleaners.

Chapter 8

Photo-Identification as a Tool for Studying Cleaner-Client Interaction Dynamics

A version of this chapter is in preparation for up to two manuscripts for journal submission.

ABSTRACT

The beneficial interactions between species, known as mutualisms, are inherently complex and dynamic, varying spatio-temporally. Given that mutualisms are essentially driven by one individual interacting with another at a specific time point, individual level behaviours of partners could have profound consequences on the resulting mutualism dynamics. It is however currently difficult to ethically discriminate between individual partners. Photo-identification could thus provide a novel, non-invasive tool, for studying individual level behaviours of mutualistic partners, by utilising between-individual variations in natural body markings. Hence, this chapter aimed to demonstrate for the first time, whether photo-identification can be applied to studies investigating the dynamics of the iconic cleaner-client mutualism. Here it was shown that photo-identification could discriminate between conspecifics of two Caribbean filefish client species (scrawled, *Aluterus scriptus* and whitespotted, *Cantherhines macrocerus*) using their body patterns (spot and saddle patch patterns respectively). Individuals of these species were observed visiting the coral head cleaning stations of the predominant Caribbean cleaner fish, the sharknose goby (*Elacatinus evelynae*) and individual filefish differed from their conspecifics in their cleaning (frequency and duration) solicitation poses, received cleaning behaviour and station visitation patterns. Client species abundance and diversity can drive cleaning interaction patterns, and here this chapter provides evidence that between-individual differences within client species may also have important implications for studies of cleaning dynamics. Overall, this chapter illustrates that photo-identification is a promising tool for studies of reef fish, particularly those investigating population

sizes and dynamics, space and habitat use, and inter- and intra-specific behavioural interactions.

INTRODUCTION

Mutualisms occur when two species beneficially cooperate (Bronstein 2015). These interactions are fundamental for structuring the ecological communities within which they occur, since they connect multiple species (Floeter et al. 2007; Sazima et al. 2010; Quimbayo et al. 2018), and also provide community benefits (e.g. pollination, Landry 2012, and parasite removal, Clague et al. 2011b; Waldie et al. 2011). Nearly every animal on the planet is connected directly or indirectly through mutualisms, yet despite their common occurence, and their ecological importance, there is limited understanding of the large spatio-temporal variations and heterogeneous interaction patterns that are observed across and within interactions (Herrera 1988; Billick and Tonkel 2003; Cheney and Côté 2005). Traditionally mutualistic studies tend to focus on population patterns, which assumes all conspecifics are the same, or that variation is random (Bolnick et al. 2011). At their simplest level however, these interactions are structured by two individuals directly interacting, so the traits and behaviours of one partner will influence the traits and behaviours of the other (Wolf and Weissing 2012): individual variations in behaviour can thus create heterogeneous mutualistic patterns at the population level (**Chapter 7**). Investigating how individual partners influence mutualism outcomes will help to clarify the dynamics and hence evolution of mutualisms under natural conditions.

It is well established that trait variations within species can influence ecological dynamics (Bolnick et al. 2011), but ethically distinguishing between individuals can be challenging (Silvy et al. 2012). Many studies have marked individuals using methods such as subcutaneous chemical markings, tattoos, tissue removal, tags and external colourants (Silvy et al. 2012; Dala-Corte et al. 2016). These invasive methods for distinguishing between individuals are not suitable for mutualistic studies however, since many of these interactions (e.g. cleaner-client and plant-pollinator) are instigated by visual signals between partners (Becker et al. 2005; Cheney et al. 2009; Leonard et al. 2011). Thus, external markings may have implications on the interaction dynamics: indeed, markings have been shown to modify animal behaviour (Murray and Fuller 2000). Instead, photographic identification offers a promising non-invasive tool for studying long-term mutualism dynamics at an individual level, since it means individuals can be followed over space and time (Bolger et al. 2012). This increasingly popular technique, uses natural variations in body marking patterns (e.g. spot and contour patterns) to distinguish between conspecifics (Bendik et al. 2013), and has been successfully used to monitor the movement patterns, population dynamics and social behaviour across terrestrial (Bolger et al. 2012) and marine mammals (Karlsson et al. 2005; Mann et al. 2012), birds (Williams and Thomson 2015), reptiles (Reisser et al. 2008), amphibians (Bendik et al. 2013) and fish (Marshall et al. 2011; Dala-Corte et al. 2016). Photo-identification has not yet, however, been applied to the study of mutualism dynamics but could further understanding on how different individuals spatio-temporally interact.

Photo-identification could be used to study the dynamics of the iconic cleaner-client mutualism. This interaction, which ubiquitously occurs on coral reefs (White et al. 2007), involves a cleaner removing ectoparasites and other material from the bodies of a client host (Feder 1966). Even within the same location, these cleaning interactions are highly dynamic and heterogeneous (**Chapters 5** and **6**; Sazima et al. 2000; Whiteman and Côté 2002b) and individual level behaviours of the cleaners can regulate interaction outcomes, influencing who interacts with whom and how (**Chapter 7**; Wilson et al. 2014). Little attention is however given to the clients, despite their solicitation posing behaviours (Côté et al. 1998; Arnal et al. 2001) and visitation patterns (Bshary and Schäffer 2002) also driving interaction dynamics (**Chapter 6** and **7**). Posing involves a client presenting their body to the focal cleaner, which signals their intent to engage in the interaction (Feder 1966). Cleaners can interact with a large diversity of clients on a daily basis (up to 132 different species, Grutter and Poulin 1998b) and the clients species identity can influence how it is cleaned and engages in the interaction (**Chapters 6** and **7**; Côté et al. 1998). However, the consequences of within-species differences in client identity on cleaning patterns is not clear. Given that coral reef fish species are renowned for their coloration and patterning (Puebla et al. 2007), and photo-identification can function by discriminating conspecifics based on pattern variations (Bolger et al. 2012), photo-identification may provide a means to determine how individual client behaviour influences the dynamics of cleaner-client interactions *in situ*. Except for some shark species (e.g. whale sharks, *Rhincodon typus,* see Arzoumanian et al. 2005 and manta rays, *Manta alfredi,* see Marshall et al. 2011), no study has used natural body markings and photo-identification to distinguish between individual reef fish; one study has however used scars to distinguish between individual goliath grouper (*Epinephelus itajara,* see Giglio et al. 2014) and pattern discriminations between individual conspecifics have been previously noted (Levy et al. 2014).

This chapter aims to demonstrate for the first time, whether client photo-identification can be applied to studies investigating cleaner-client interaction dynamics. Body pattern based photo-identification is reliable if focal animals have at least two distinct colours on specific areas of their bodies (Buray et al. 2009) and if the chances of stable pattern duplication is low (governed by a complex pattern and small population size, Pennycuick 1978). It was thus hypothesised that individuals from the filefish family (Monacanthidae) may provide ideal candidates to test whether photo-identification can be used to distinguish between conspecific reef fish. Using video data, *in situ* cleaning interactions were observed between the predominant Caribbean cleaner fish, the sharknose goby (*Elacatinus evelynae*), and two Monacanthidae reef fish clients: the whitespotted filefish (*Cantherhines macrocerus*) and the scrawled filefish (*Aluterus scriptus*). These free-ranging species are common clients of the sharknose goby (**Chapter 5**). Whitespotted filefish are characterised by a white pigment saddle patch and orange body, whilst scrawled filefish are tan in colour and can be identified from a large number of small black spots and blue lines across their bodies (Humann and Deloach 2014). Given that saddle patch shapes and spot patterns have been previously used to distinguish between individuals within other marine species (saddles: pilot whales, *Globicephala melas,* see Auger-Méthé and Whitehead 2007, spots: whale sharks, Arzoumanian et al. 2005, gray seals, *Halichoerus grypus*, see Paterson et al. 2013), it was predicted that individual filefish could be distinguished from conspecifics using these markings. Following identification, it was then determined whether photo-identification could be applied to cleaner-client interactions by investigating differences in the cleaning and posing behaviour (frequency and duration) of individual filefish. It is well established that within many animal species, including fish, individuals within populations can differ in their parasite burdens (Crofton 1971), hence individual clients are likely to vary in their nutritional value to the cleaner. It was hypothesised that filefish individuals would differ in their received cleaning behaviour (both durations and frequency), a pattern also reflected across client species, due to asymmetric parasite loads (Soares et al. 2007). Parasite infection can also influence a client's tendency to seek out cleaning (Grutter 2001). Posing, however, incurs an energetic cost which increases with time spent posing, and thus just like the patterns observed across species (Côté et al. 1998), it was expected that individuals would differ in their posing behaviours (frequency and duration). Finally, cleaning patterns can differ spatially between cleaning stations, which are topological features occupied by cleaners who wait for visiting clients (Potts 1973a; Mills and Côté 2010). Individual clients do re-visit more favourable cleaning stations, but these observations are temporally limited (e.g. to a single 60 minute dive, Bshary and Schäffer 2002; Soares et al. 2008b). It was thus also assessed whether individual filefish showed non-random station visitations and whether they showed site fidelity to cleaning stations over a longer time scale (6 weeks).

METHODS

Photograph collection

Whitespotted (*Cantherhines macrocerus*) and scrawled filefish (*Aluterus scriptus*) images were collected from Booby Reef, Man O' War Bay, Tobago (11°19.344′N 060°33.484′W) over a six week period in May – July 2017 using underwater video cameras. Booby Reef can be considered as a relatively degraded fringing reef which extends to Booby Island, located 85 – 90 m northeast from the shore (Ramsaroop 1982). The nearshore study area (1 – 2 m deep covering an area of 70 m x 60 m) is primarily composed of algae covered dead coral and living brain corals (Faviidae). Sharknose gobies (*Elacatinus evelynae*) are the predominant cleaner species on this reef, occupying coral cleaning stations. Cleaning stations (n = 82) within this study area have been monitored annually as part of a long-term study (8 year, 2010 – 2017) and are located at least 1 m apart from one another. Whitespotted filefish have been observed on Booby Reef across all 8 years of long-term study, whilst scrawled filefish have been seen in 7 years, and both are often observed as clients of gobies (**Chapter 5**).

To capture filefish photographs, video cameras were placed (n = 10 QUMOX SJ400 Action Cams, as in **Chapter 3**) at 27 of the marked cleaning stations across the study period (n = 192 videos, mean ± S.E. number of videos per station = 7.11 ± 1.26). Cameras were placed daily and only during daylight hours (between 07:00 and 17:30) and were left to record for the full extent of their battery lives (max video length =

7189 seconds, minimum length = 80 seconds, mean ± S.E. video length = 5410.59 seconds ± 115.38). Cameras were positioned so that a goby cleaner was initially in view; however, cleaners often traverse around their large coral heads (ca. 1 - 2 m^3) and thus could be out of view from fixed cameras at times. Cameras were placed on the same side of the cleaning station each time. The exact location of the camera in terms of the angle towards, and distance from the station was however, not standardised within and across stations (mostly due to the position of cleaners on the coral, the size of the coral head, the conditions on the reef (in terms of swell), and inter-observer differences). Some of these sharknose goby stations (n = 6) were also occupied by a second cleaner species, the French angelfish (*Pomacanthus paru*), which only clean clients when juvenile (Sazima et al. 1999): the presence of this species at stations was also noted.

Filefish photographs were obtained through screen captures from video footage (n = 192 videos, total observation time = 12 days, 33 minutes and 53 seconds). Filefish photos were taken with the individual as close to a lateral position as possible to the camera. Only filefish that were observed interacting with the station (through posing and/or cleaning, n = 236 visits) were recorded. Like many reef fish (e.g. *Chaetodon paucifasciatus*, see Levy et al. 2014), filefish body patterns are not the same on both sides, and thus where possible photos were obtained of the visiting filefish's right and left side. Whitespotted filefish individuals can also express a white spotted body pattern rather than a saddle, which they can rapidly switch between (Figure 8.1, Randall 1964). Individuals can also rapidly change the expression of each spot (Allen et al. 2015) meaning that spot patterns could also vary within individuals as well as across individuals. Thus, individual whitespotted filefish were not identified whilst they were expressing their spot phase, and instead photographs of these fish were only taken when saddles were observed.

Identifying individual filefish

I. Whitespotted

Whitespotted filefish were identified using the shape and size of their saddle patch (Figure 8.1).

Whitespotted filefish - *Cantherhines macrocerus*

Scrawled filefish - *Aluterus scriptus*

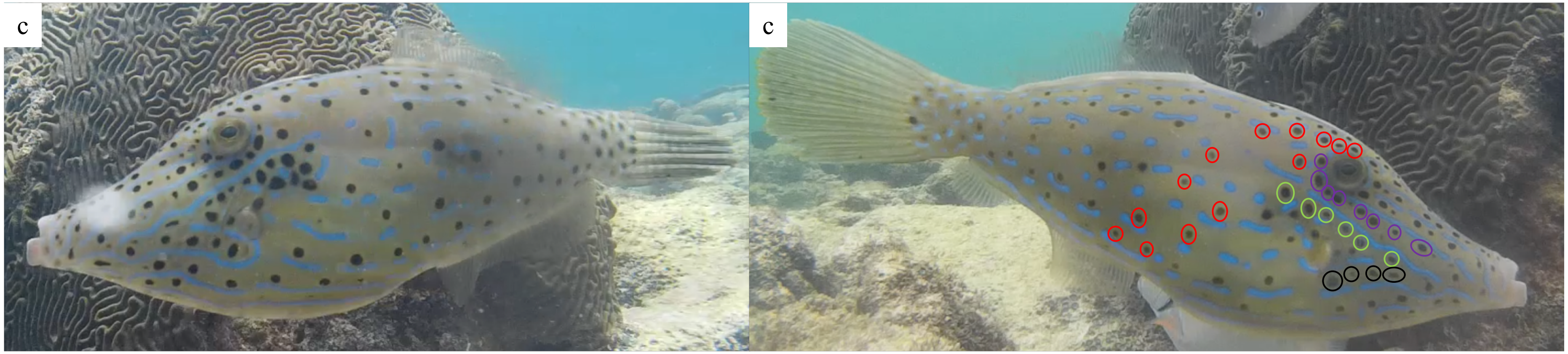


Figure 8.1: Images illustrating how individual whitespotted (*Cantherhines macrocerus*) and scrawled filefish (*Aluterus scriptus*) can be distinctly identified from conspecifics. Letters represents different individuals. Both (a) and (c) demonstrate differences in body patterning between left and right sides of filefish. Arrow on (b) points to two spines on base of whitespotted caudal fin, (b) shows individual transitioning between saddle and spot phase. The relative area of the whitespotted saddle was calculated for each individual (shown on individual b), whilst four spot areas (illustrated on c) were used to distinguish between individual scrawled filefish.

Although many studies use photo-identification software to identify individuals through natural body markings (e.g. van Tienhoven et al. 2007; Bendik et al. 2013), contour based photo-identification is sensitive to even slight differences in photo angles, leading to identification errors (Caci et al. 2013). Given that it was not possible to exactly standardise the angle of the filefish and there were a relatively small number of whitespotted filefish visits (n = 183) to analyse, filefish individuals across observations were matched by eye: human matching has similar success and accuracy to computer-based matching at small sample sizes (Speed et al. 2007).

Whitespotted filefish have two spines at the base of their caudal fin (Figure 8.1), and the size of these spines can vary between individuals (Randall 1964). Prior to individual identification, photographs of whitespotted filefish were first grouped by the size of these spines (large, medium and small, executed by two observers). Following this, the area of body over which the saddle patch occupied was calculated (*see below*). Individual filefish with similar saddle patch areas and spine sizes were then compared by eye for individual matches. This method follows the computer-based 'Interactive Individual Identification System (I^3S)' whereby the programme groups individuals using similar areas and the final matches are made by human eye and judgement (van Tienhoven et al. 2007). All photographs were independently re-matched three times by the same observer to check consistency, and another independent observer verified final matches.

To quantify the proportion of the body over which the saddle occupied, the border of saddles were drawn around and the area computed using the freehand tool in ImageJ (Schneider et al. 2012). To distinguish between individuals, since there was no scalar in the images, saddle areas were compared to a standardised five-sided polygon created on the body using straight-lines between the individual's fins, mouth and eye (Figure 8.1). Thus, the proportion of the polygon over which the saddle occupied was compared between individuals. By using a pentagon, it was possible to account for, to some extent, the filefish's body angle to the camera: commonly used photo identification software (e.g. I^3S) only allows three reference points (van Tienhoven et al. 2007) and is thus more prone to angle distortion. However, filefish body angle did still distort area proportions which is why this method was not relied on solely for matching. This quantification process was completed three times for each picture to account for any errors in free-hand drawing.

II. Scrawled

Individual scrawled filefish were identified using their black spot patterns, and where visible, their blue scrawled lines. Scrawled filefish patterning is made up of a high number of spots (Figure 8.1), and so to facilitate individual matching, the spot patterns in four areas on the body were considered; three places under the eye and the patch between the eye and dorsal/anal fin (shown on Figure 8.1). Although it was possible to use photo-identification software to distinguish between scrawled filefish individuals, the small number of different patterns and visits (n = 53 scrawled visits), meant it was a task that could easily and confidently be done by judgement and eye. Photo-identification by eye is an acceptable and manageable method for small numbers of photographs; the process becomes insufficient and unreliable when working with increased sample size, necessitating an automated matching system (Arzoumanian et al. 2005). Scrawled filefish visits were assigned to IDs independently three times, and all scrawled filefish matches were verified by an independent observer.

It was not always possible to identify individual scrawled and whitespotted filefish due to the lighting, individuals body angle towards the camera, and missing information (e.g. body not fully in view or whitespotted just showing spot phase). These visits were assigned as unidentifiable.

Applying photo-identification to filefish-cleaner fish interactions

To determine whether filefish photo-identification can be applied to studies investigating the dynamics of cleaner-client interactions, the durations and frequencies of cleaning of, and posing by, filefish in each cleaning station visit were recorded. These data were then linked to the individual's assigned ID. Videos were analysed following a 20 s period from when an observer placed the camera to reduce the effect of disturbance on filefish visitations. Filefish-cleaner interactions were limited to the side of the cleaning station the camera was facing (cleaning stations are ca. 1 – 2 m^3), thus this likely captured conservative durations and frequencies of cleaning and posing behaviours since it was unknown what was happing on the other side of the station.

Data analysis

Data were analysed similarly to in **Chapter 5** using R version 3.4.3 (R Core Team 2017) and were considered separately for scrawled and whitespotted filefish.

For all analyses, individuals were only included if they were observed more than once (meaning n = 4 scrawled filefish were excluded, final sample sizes: whitespotted = 9 individuals, scrawled = 10 individuals). To investigate patterns of filefish cleaning, two cleaning and posing measures for each individual were calculated; cleaning/posing frequency and cleaning/posing duration. For each filefish visit, it was binary assigned as to whether the individual was cleaned or not. Given that individuals differed in the number of times they were observed visiting cleaning stations, cleaning frequencies thus represented the proportion of visits each individual was observed being cleaned. Cleaning durations represented the time spent cleaning per visit (total clean duration / number visits). The same method was used to calculate a posing frequency and duration measure for each identified filefish. Due to the nature of the static video cameras, it was not always possible to gain duration data for each visit: posing and/or cleaning may have started and/or ended out of view. Thus, these visits were not included in individual filefish duration calculations but were considered in frequency data. Likewise, it was sometimes not possible to determine whether a filefish was posing or being cleaned, so these visits were excluded from analyses.

To determine whether any filefish individual was cleaned and/or posed more or less than their conspecifics (both frequency and duration), four z-scores for each individual were calculated (frequency data were log10 transformed to increase the data resolution for comparison). Z-scores were calculated from bootstrapped (10,000 resampling) means and standard deviations. Individual filefish with z-scores greater than 1.96 were considered to have high cleaning/posing behaviours, whilst individuals with z-scores less than -1.96 were considered to receive low cleaning and/or low posing tendencies. Spearman's correlations determined whether there was a significant relationship between posing/cleaning frequencies and posing/cleaning durations. All Spearman's p-values were adjusted for multiple testing using Benjamini-Hochberg corrections.

Videos cameras were placed at 27 marked cleaning stations over the study period. To investigate filefish station visitation patterns, the proportion of stations (out of 27) visited for each filefish individual was first calculated. A random data set of 10,000 'filefish' individuals was also created using re-sampling of the same 27 cleaning stations and the number of visits randomly ranging from two to 20 (two whitespotted filefish were re-sighted 20 times across stations). From this simulated data set, the proportion of stations visited for each row was also calculated. To determine whether individual filefish visited fewer cleaning stations than random, z-scores were calculated for each individual using the mean and standard deviations from the proportion of stations visited across the random data. Z-scores < -1.64 (representing a one-tailed $p < 0.05$) indicated filefish that visited significantly fewer cleaning stations than random. No individual filefish had a z-score > 1. Pearson's Chi-squared tests with Yate's continuity correction were used to determine whether filefish interacted significantly more frequently with multi-species (occupied by sharknose gobies and French angelfish, *Pomacanthus paru*) or single-species (just occupied by sharknose gobies) stations.

To determine whether individuals showed site fidelity/preference towards certain stations (represented by an unusually high number of visits compared to the other marked stations), the probability of each filefish visiting each station was calculated. Station visitation probability values were also calculated for each randomly generated row (representing an 'individual') in the simulated data. All probability values were subsequently log10 transformed. Two scrawled filefish individuals had the same probability of visiting each station (probability value = 0 per station) and thus were automatically considered to show no site fidelity. To identify whether other individuals showed significant site fidelity, the relative standard error (RSE) was calculated across log10 transformed station visitation probabilities for each filefish individual and randomly generated data row. The RSE (expressed as a %) is comparable to the coefficient of variation (CV) but instead provides a measure of variability whilst accounting for the mean and sample size (individuals differed in the number of stations they visited). A high RSE meant that an individual was not consistent in their station visitation behaviour, i.e. showing preference for certain station(s) over others. Z-scores for each individual filefish and their RSEs were subsequently calculated using a mean and standard deviations generated across RSEs of the random data set. To determine whether different filefish individuals differed from the conspecifics in the strengths of their site fidelity, z-scores were calculated from bootstrapped mean and standard deviation (10,000 resampling from real data RSEs) and it was determined which individuals had z-scores > 1.96 (strong site fidelity to certain station) or scores lower than -1.96 (weaker site fidelity to certain station).

Finally, Spearman's rank tests determined whether individual whitespotteds' site fidelity to cleaning stations linked with their cleaning and posing behaviour: individual's probability of visiting each station correlated with their posing and cleaning, frequencies and durations (four correlations for whitespotted: cleaning and posing frequency/duration, n = 34 across 9 individuals). Scrawled filefish only differed in their posing durations (only one individual was observed being cleaned, and all individuals had the same posing frequency), so only one correlation was used for this species (posing duration versus probability of visiting station, n = 14 across 7 individuals).

RESULTS

Can individual filefish be identified using photo-identification?

Individual filefish could be identified using photo-identification. Across 192 videos totalling 12 days, 33 minutes and 53 seconds of observation time, 236 instances of filefish interacting with cleaning stations were recorded (n = 183 whitespotted, n = 53 scrawled). Filefish were observed in 43.8% of videos (whitespotted observed in 79 videos, scrawled in 30 and both species observed in 15 videos). From these observations, nine individual whitespotted filefish were identified (using saddle shape and size, Figure 8.2) along with 14 scrawled filefish (using spot patterns, Figure 8.2). For four scrawled individuals, only their left side was observed, and two different individuals were also documented only using their right side. Thus, up to 16 individual scrawled filefish could have been observed altogether (only 14 individuals were included in analyses). Overall 168 (out of the 236) filefish observations could be assigned to individual IDs.

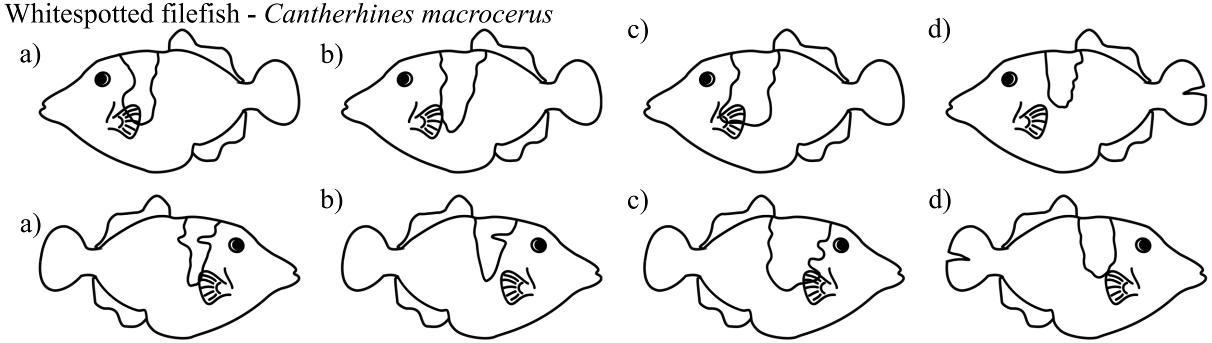
Individual scrawled filefish were more successfully identified compared to whitespotted: 86.8% of scrawled were assigned one of 14 identities (7 out of 53 visits not assigned), whilst 66.7% of whitespotted visits were assigned to one of nine identities (61 out of 183 not assigned). The primary reason for unsuccessful identification was due to poor lighting (e.g. glare in 38.2% of cases), whilst 29.4% of individuals could not be identified due to a lack of body marking information (e.g. body not fully in view or whitespotted filefish showing their spot phase). The remaining individuals could not be identified due to poor image quality (10.3%), their body angle being too perpendicular to the camera (10.3%) or a combination of factors (11.8%).

Can photo-identification be applied to studying cleaner-client interactions?

I. *Cleaning patterns*

From 23 identified filefish individuals (n = 14 scrawled, n = 9 whitespotted) and their 168 combined visits to cleaning stations (in 94 videos), 56 cleaning and 135 posing events were observed. Whitespotted filefish individuals were cleaned 48 times and were documented to pose 96 times. Across these events, there was significant between-individual differences in whitespotted filefish posing and cleaning behaviour ($p < 0.05$, based on z-scores from bootstrapped 10,000 means and standard deviations, Table 8.1). Some individuals had significantly higher or lower posing/cleaning tendencies compared to conspecifics and they also differed in their time spent posing and/or cleaning; individuals posed/were cleaned for significantly longer or shorter durations (on average) than other conspecifics (Table 8.1, highlighted in dark grey for high values and light grey for low values). Individuals that received a lower frequency of cleaning were also cleaned for shorter durations per visit (Spearman's rho = 0.85, $p = 0.004$).

Whitespotted filefish - *Cantherhines macrocerus*



Scrawled filefish - *Aluterus scriptus*

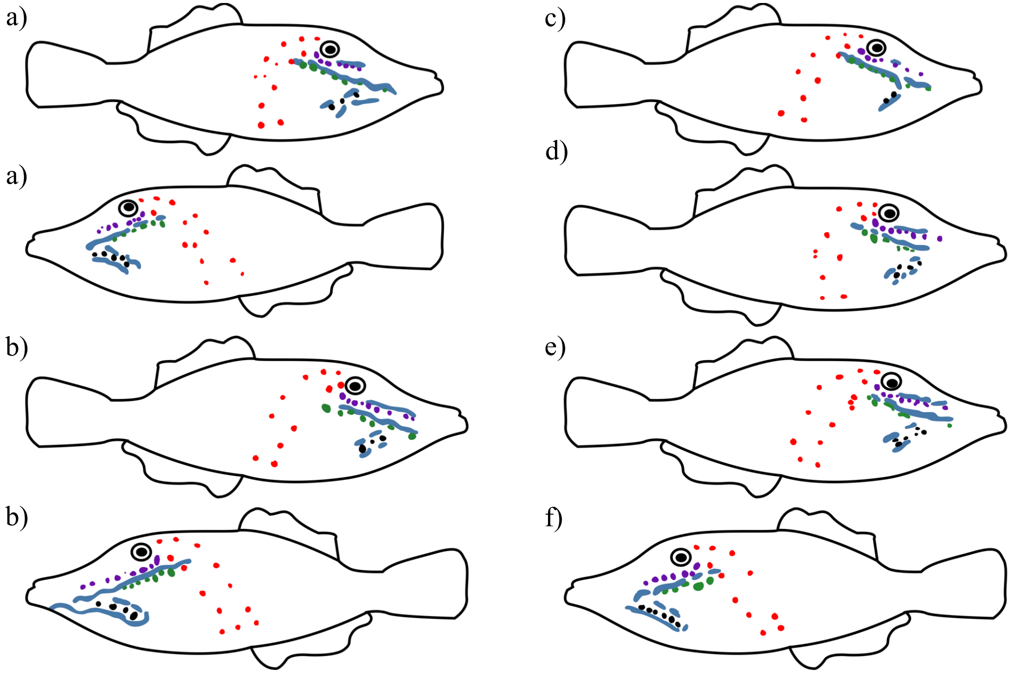


Figure 8.2: Example individual whitespotted (*Cantherhines macrocerus*) and scrawled filefish (*Aluterus scriptus*) identified from Booby Reef, Tobago in the summer of 2017. Individual whitespotted were identified through saddle shapes and sizes, whilst scrawled individuals were identified using four spot patterns. Left and ride side patterns/spots differ within individuals. Letters represents different individuals within each species.

Table 8.1: Cleaning and posing behaviour (frequency and duration, seconds) of individual whitespotted filefish (*Cantherhines macrocerus*) visiting up to 27 marked cleaning stations on Booby Reef, Tobago. Table shows the number of re-sightings of each individual and the number of videos and stations each individual was observed in/at. Posing/cleaning tendency represents the percentage of visits where posing/cleaning was observed, whilst posing/cleaning durations per visit represent the mean ± S.E. time spent posing/being cleaned during each visit. Individual whitespotted filefish showing significantly higher values to conspecifics are highlighted in dark grey whilst significantly lower values are illustrated using light grey ($p < 0.05$).

ID	Re-sightings	Videos sighted in	Cleaning stations	Posing tendency	Cleaning tendency	Posing duration/visit	Cleaning duration/visit
WSFF_1	18	13	3	85.7%	64.3%	10.2s ± 2.9	12.7s ± 3.2
WSFF_2	20	11	2	87.5%	68.8%	17.7s ± 3.5	18.9s ± 6.5
WSFF_3	16	5	3	100%	15.4%	5.1s ± 1.0	0.8s ± 0.8
WSFF_4	10	5	4	90.0%	20.0%	6.4s ± 2.4	3.6s ± 3.4
WSFF_5	11	10	6	100%	33.3%	8.0s ± 2.6	5.8s ± 4.7
WSFF_6	20	13	6	94.7%	36.8%	27.4s ± 8.2	7.7s ± 3.7
WSFF_7	15	12	5	85.7%	64.3%	13.6s ± 5.0	16.2s ± 4.8
WSFF_8	8	7	4	71.4%	57.1%	25.1s ± 13.7	5.0s ± 3.8
WSFF_9	4	2	2	100%	25.0%	6.0s ±1.2	11.3s ± 11.3

This study begins to show differences in cleaning and posing patterns between individual whitespotted filefish, and albeit the small re-sighting sample size, only one of the 14 scrawled filefish was cleaned across observations (cleaning observed in 21% of scrawled filefish visits all for SFF_1, SFF_1 clean tendency = 57.1%, mean duration ± S.E. = 33.2s ± 11.3). Despite this lack of cleaning, all scrawled filefish individuals were observed posing each time they visited a cleaning station (all individuals posing tendency = 100%), but there were significant between-individual differences in mean posing durations per visit (Table 8.2, $p < 0.05$, based on z-scores from bootstrapped 10,000 means and standard deviations): two individuals posed for significantly longer than the remaining 10 conspecifics, whilst four of these individuals posed for shorter durations ($p < 0.05$, Table 8.2).

Table 8.2: Cleaning and posing behaviour (frequency and duration, seconds) of individual scrawled filefish (*Aluterus scriptus*) visiting up to 27 marked cleaning stations on Booby Reef, Tobago. Table shows the number of re-sightings of each individual and the number of videos and stations each individual was observed in/at. Posing durations per visit represent the mean ± S.E. time spent posing during each visit. Individual filefish showing significantly higher values to conspecifics are highlighted in dark grey whilst significantly lower values are illustrated using light grey ($p < 0.05$). Only those individuals sighted on more than one occasion were used in analyses ($n = 10$). Data for individuals not included in analyses are bracketed. Some durations were not available as behaviour started out of view or visits could not be defined as posing or cleaning – hence missing values even where re-sightings greater than one.

ID	Re-sightings	Videos sighted in	Cleaning stations	Posing duration/visit
SFF_1	15	4	3	50.0s ± 10.5
SFF_2	1	1	1	(18s ± NA)
SFF_3	3	3	2	25.3s ± 19.3
SFF_4	1	1	1	(3s ± NA)
SFF_5	5	5	4	20.3s ± 8.9
SFF_6	1	1	1	(14s ± NA)
SFF_7	3	1	1	NA
SFF_8	3	3	3	4.7s ± 0.9
SFF_9	3	3	2	29s ± 8.7
SFF_10	2	2	1	6s ± NA
SFF_11	2	1	1	9.5s ± 1.5
SFF_12	2	2	2	7s ± NA
SFF_13	2	2	1	3s ± NA
SFF_14	1	1	1	(5s ± NA)

II. Station visitation patterns

Filefish were observed visiting 22 of the 27 videoed cleaning stations (only 19 stations by individually identified filefish, Figure 8.3). The number of stations each individual interacted with ranged from one to six (Tables 8.1 and 8.2, Figure 8.3). Across the 19 identified filefish, 13 showed non-random station visitation patterns ($p < 0.05$, Figure 8.3, nine out of 10 scrawled, four out of nine whitespotted, based on z-scores less than -1.64 calculated using mean and standard deviation of proportion of 27 available stations visited (log10 transformed) across 10,000 randomly simulated data). Six of the 22 visited sharknose goby (*Elacatinus evelynae*) stations were also occupied by the facultative cleaner, the juvenile French angelfish (*Pomacanthus paru*), and whitespotted filefish interacted with these multi-species cleaning stations more frequently than they did with single species sharknose goby stations (Figure 8.3, across all individuals n = 183 visits, Pearson's $\chi^2_1 = 8.31$, $p = 0.004$, across identified individuals n = 122 visits, Pearson's $\chi^2_1 = 8.39$, $p = 0.004$). Scrawled filefish did not interact with these station types differently (Figure 8.3, across all individuals n = 53 and across identified individuals n = 46, both $p > 0.02$).

For individual filefish visiting more than one station, only two scrawled filefish and nine of the ten whitespotted filefish showed significant site fidelity to a particular station(s) ($p < 0.05$, Figure 8.3, based on z-scores greater than 1.64, generated from RSE mean and standard deviations calculated across 10,000 randomly simulated data sets). For both whitespotted and scrawled filefish, individuals differed from conspecifics in the strength of their site fidelity (Figure 8.3, $p < 0.05$). Some individuals showed strong site fidelity, illustrated by a significantly high relative standard error value (RSE), whilst others had weaker site fidelity (low RSE values, Figure 8.3, compared using z-scores from bootstrapped 10,000 means and standard deviations within scrawled and whitespotted populations).

The home range sizes of both filefish species are unknown, so it was not possible to determine whether filefish site fidelity is simply down to the station locations on the reef. However, whitespotted individuals were less likely to pose at stations which they visited more frequently (Spearman's rho = 0.53, $p = 0.005$ adjusted for multiple testing, $p = 0.001$ before corrections). There was also a negative trend between whitespotted cleaning duration and the probability of visiting the station (Spearman's rho = -0.36, $p = 0.073$, $p = 0.036$ before corrections).

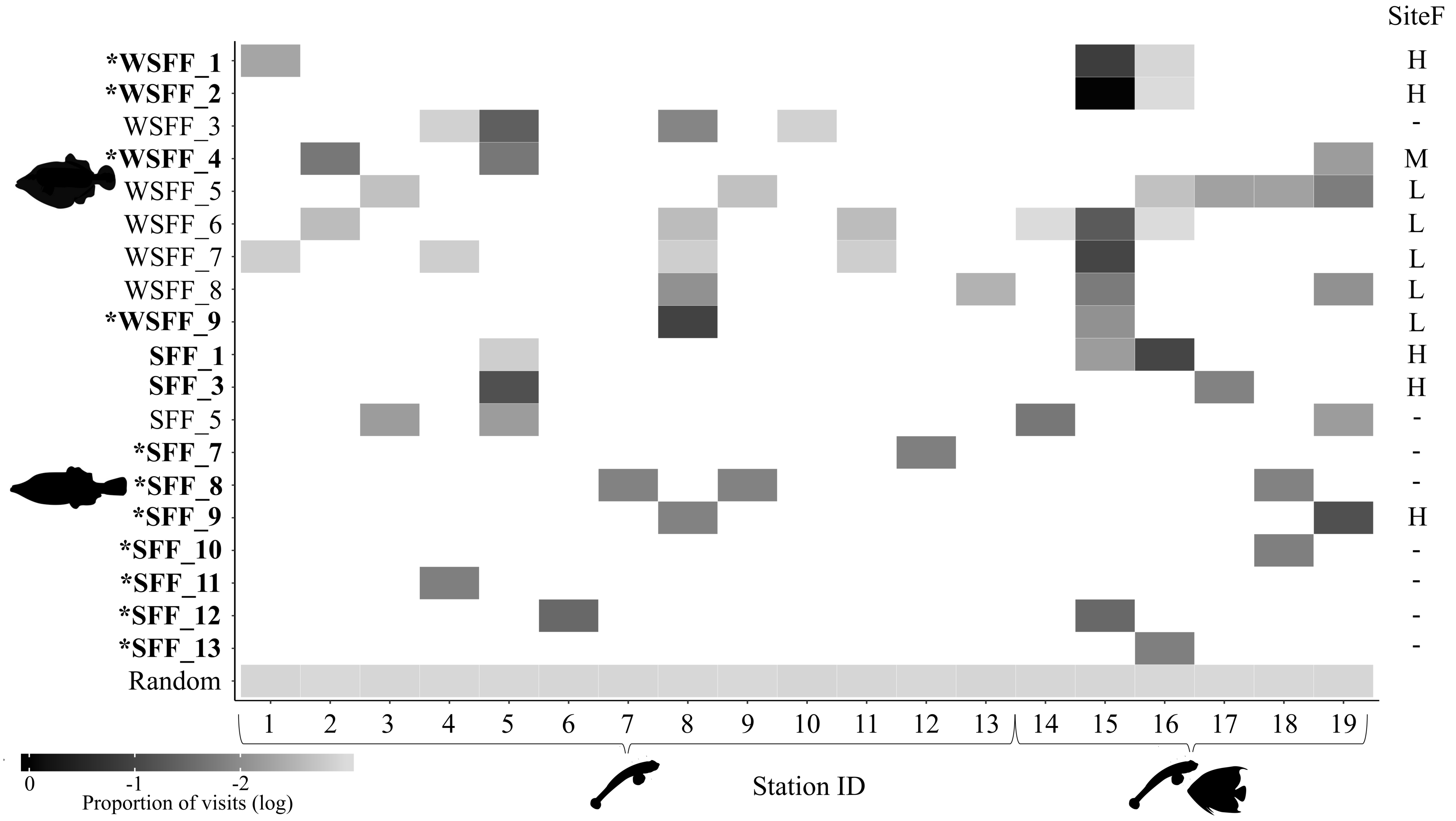

SiteF
*WSFF_1 H
*WSFF_2 H
WSFF_3 -
*WSFF_4 M
WSFF_5 L
WSFF_6 L
WSFF_7 L
WSFF_8 L
*WSFF_9 L
SFF_1 H
SFF_3 H
SFF_5 -
*SFF_7 -
*SFF_8 -
*SFF_9 H
*SFF_10 -
*SFF_11 -
*SFF_12 -
*SFF_13 -
Random
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Station ID
0 -1 -2
Proportion of visits (log)

Figure 8.3: Cleaning station visitation patterns of whitespotted (*Cantherhines macrocerus*) and scrawled filefish (*Aluterus scriptus*) on Booby Reef, Tobago. Darker colours show an increased proportion of station visits (log10 transformed). Visitation proportions for each station were also calculated from a 10,000 simulated data set to represent random visitation and is included for reference. Individuals highlighted in bold and showing * visited fewer stations than expected by random. Stations could be grouped as to whether they were only occupied by a sharknose goby (*Elacatinus evelynae*) or occupied by both a sharknose goby and a juvenile French angelfish (*Pomacanthus paru*). Eleven individuals showed significant site fidelity towards stations (identified using letters H, M and L) and within species, individuals differed from conspecifics in their levels of site fidelity towards cleaning stations (H = z-score > 1.96, M = z-score -1.96 to 1.96, L = z-score < -1.96). Four scrawled individuals were not included here as they were only observed once (Table 8.2).

DISCUSSION

This chapter demonstrates that photo-identification is a useful non-invasive tool for identifying conspecific reef fish and can be applied to studies of cleaner-client interactions. Individual scrawled (*Aluterus scriptus*) and whitespotted (*Cantherhines macrocerus*) filefish could be distinguished from conspecifics using their natural body patterning (spots – scrawled, saddle – whitespotted): across 6 weeks, nine individual whitespotted and 14 individual scrawled individual filefish were identified and repeatedly observed on the same reef in Tobago. Subsequently, through identifying individual filefish clients visiting sharknose goby (*Elacatinus evelynae*) cleaning stations, this work also revealed that individual filefish differed in their posing behaviour at stations, and some individuals received more cleaning, on average, than others. There were also between individual differences in cleaning station visitation patterns: individual filefish non-randomly interacted with certain stations and there was evidence of station site fidelity. Together, photo-identification could provide a useful tool for reef fish studies, particularly those investigating population dynamics, space and habitat use, and inter- and intra-specific behavioural interactions.

Here, photo-identification provided a means to discriminate between individual reef dwelling fish over time and space: this method has only been used previously to identify larger individuals from elasmobranch fish species that can traverse sizeable distances across and between reefs (e.g. lemon sharks, *Negaprion brevirostris*, see Buray et al. 2009, manta rays, *Manta alfredi,* see Marshall et al. 2011, whale sharks, *Rhincodon typus,* see Arzoumanian et al. 2005). Although this work only uses photo-identification to discriminate between conspecifics of two filefish species, it is not to say that this method cannot be applied to other species in the Caribbean and on other reefs worldwide. On coral reefs, the diversity of colours and complex body patterning of fish (Puebla et al. 2007) should support the use of photo-identification (Pennycuick 1978; Buray et al. 2009), and body pattern variations between conspecifics inhabiting the same reef have been previously noted for other reef species (e.g. bands of crown butterflyfish, *Chaetodon pauifasciatus*, see Levy et al. 2014, UV facial patterning of ambon damselfish, *Pomacentrus amboinensis,* see Siebeck et al. 2010). Following on from the initial work presented here, it is thus hypothesised that this non-invasive method could be used to distinguish between conspecifics from other reef fish species (e.g. Caribbean: tail stripes of striped parrotfish, *Scarus iseri,* yellow body patterning of French grunt, *Haemulon flavolineatum*, Indo-Pacific: band shape and size of Orangeband surgeonfish, *Acanthurus olivaceus*, face pattern of checkerboard wrasse, *Halichoeres hortulanus*, band patterning of spot-banded butterflyfish, *Chaetodon punctatofasciatus*).

One of the common uses of photo-identification is to non-invasively estimate population sizes using mark-recapture methods (e.g. humpback whales, *Megaptera novaeangliae*, see Smith et al. 1999, giraffes, *Giraffa camelopardalis tippelskirchi*, see Bolger et al. 2012). Although it was not possible to estimate population sizes of filefish here due to study design (i.e. cameras being placed at specific cleaning stations, rather than at stratified locations across the reef), nine whitespotted and 14 scrawled filefish were identified using photo-identification. This small number of recognised individuals within each species perhaps suggests that the filefish populations on this study reef may only be made up of only a handful of individuals. Both scrawled and whitespotted filefish are usually found on the reef on their own or in pairs (Froese and Pauly 2018) and thus it is perhaps unsurprising that few individuals could be identified over this small reef area (60 m x 70 m). Nevertheless, these filefish species are regularly caught by local fishermen (*personal observation*) and thus the impact of removing a minimal number of filefish, may have large implications on small populations. In general very little is known about tropical filefish life history and behaviour, in terms of their longevity, reef use, and social and reproductive behaviour (most information is from descriptions by Randall 1964). Thus, through repeatedly observing individuals over time, photo-identification could provide a useful tool for expanding our basic knowledge on these filefish species and many other understudied reef fish.

Photo-identification also provides information on animal movement patterns, population dynamics and social behaviours (e.g. Karlsson et al. 2005; Buray et al. 2009; Marshall et al. 2011; Paterson et al. 2013; Williams and Thomson 2015) and here this chapter suggests a new application: the study of mutualism dynamics. Mutualistic patterns are driven by one individual interacting with another at a specific time point, and thus this study begins to show that photo-identification can enhance our understanding of cleaner-client interaction patterns at an individual level. Here, some filefish individuals differed in their posing behaviour towards stations whilst some individuals were cleaned more than others. Individual clients will differ in their parasite loads (Crofton 1971), which could influence their tendency to seek out cleaners (Grutter 2001) and/or nutritional value to the cleaner: received cleaning behaviour across different species can correlate with parasite assemblages (Soares et al. 2007). Posing also incurs an energetic cost, through missed foraging opportunities for example (Poulin and Vickery 1995), which will increase with time spent posing. Across all species, individuals will differ from conspecifics in their physiological state and metabolism (Toscano et al. 2016), influencing how much energy can be invested in interactions. In addition, individual clients could also consistently differ in their personality traits (e.g. bold-shy, as shown for Rocky mountain elk, *Cervus canadensis*, clients of black-billed magpies, *Pica pica* by Found 2017) which could regulate individual level cleaning patterns (as shown for cleaners in **Chapter 7**). Even with a small sample size, this work begins to demonstrate that individual clients can influence patterns of cleaning interactions. Future work should now investigate this in more detail across more individuals (like in **Chapter 7**).

The dynamics of animal populations are closely linked to the spatial arrangement and movements of individuals (Kernohan et al. 2001), and here this chapter shows that individual filefish differ in their space use patterns, visiting only certain cleaning stations and showing site fidelity to others, by re-visiting the same site over time (a result previously observed in short-term ~ 60 minute individual observations, Bshary and Schäffer 2002; Soares et al. 2008b). Given our limited knowledge of filefish behaviour, it is currently not clear why these coral sites are particularly important (e.g. habitat characteristics, home range behaviour). Indeed, interaction patterns could simply arise as a result of random movements within an individual's home range (Fagan et al. 2013). However, whitespotted filefish often visited cleaning stations also occupied by the facultative cleaner, the juvenile French angelfish (*Pomacanthus paru*). This cleaner species is considered to be a specialised cleaner attracting and interacting with a high diversity of species, many of which can be considered rare on the reef (e.g. 31 client species, Sazima et al. 1999). However, why this cleaner species is favourable remains to be determined. In addition, at an individual level, whitespotted filefish posing tendency negatively correlated with station visitation frequency. *Ex situ* the bluestreak cleaner wrasse (*Labroides dimidiatus*) can discriminate between familiar and unfamiliar conspecifics (Tebbich et al. 2002) and clients can use the visual cues of stations to visit cleaners (Huebner and Chadwick 2012). Remembering spatial locations following beneficial interactions is less cognitively demanding than recognising individuals (McAuliffe and Thornton

2015), but the cognitive abilities and memory retention for this cleaner and these client species is unknown. Investigating individual client space use over longer time periods (e.g. months and years) would be useful, especially since sharknose goby cleaners show high turnover rates on their stations (< 50 days, White et al. 2007): the lifespan of tropical filefish species is unknown, but temperate filefish have been documented to live for around ~ 3 years (*Stephanolepis* spp. using length frequency distribution and growth ring count of dorsal spine, Mancera-Rodríguez and Castro-Hernández 2004; EL-Ganainy and Sabra 2008).

Overall, this chapter demonstrates, for the first time, that photo-identification provides a means to discriminate between individual reef fish over time and space. Photo-identification is a useful tool that can inform our understanding of species population sizes and dynamics, movements patterns and habitat preferences, and has ultimately contributed to the conservation practices for many species (e.g. endangered Hector's dolphin, *Cephalorhynchus hectori*, Gormley et al. 2012). Thus, this method could help the conservation of reef fish, which are currently experiencing severe declines in their abundance and diversity as a result of anthropogenic changes (Carr et al. 2002; Munday 2004). This chapter also begins to demonstrate that photo-identification can be novelly applied to studies of mutualism dynamics. Although this work does not thoroughly investigate between-individual differences, it begins to show that individual filefish clients engage differently to conspecifics in mutualistic cleaning interactions (through posing, cleaning and cleaning station visitation patterns). Ultimately this will create heterogeneous population level patterns of the behaviour (like **Chapter 7**). The role of client species in governing cleaner client interaction patterns is often neglected, despite **Chapters 6** and **7** showing the importance of client species diversity, abundance and behaviour in maintaining the interaction. Mutualistic patterns are governed by context-dependency (**Chapter 6**; Chamberlain et al. 2014; Hoeksema and Bruna 2015) and individual variations in behaviour may be adaptive under one context, but not another (Wilson 1998). Applying photo-identification methods to studies of mutualisms may thus provide a novel means to determine which factors are consistent and important predictors of interaction patterns over time and space at an individual level, ultimately influencing population patterns of interaction outcomes.

Chapter 9

Discussion - The Dynamics and Pervasiveness of Cleaner-Client Interactions

The aim of this thesis was to investigate what factors influence the dynamics and hence occurrence of cleaning interactions across individuals, populations and species. **Chapter 2** shows that cleaning, and its parallel behaviour allogrooming, have evolved independently across three different phyla, but together the occurrence of the behaviour across animals is relatively rare. This chapter also highlights how the occurrence and dynamics of cleaning could vary within and across individuals, populations and species. **Chapters 3** and **4** also investigate the maintenance and evolution of cleaning by showing that an opportunistic facultative wrasse cleans to supplement their diet (**Chapter 3**), whilst a previously considered dedicated cleaner, can adopt a facultative strategy by replacing cleaning with coral feeding (**Cleaner 4**). The naturally stressed context in which this switched strategy was observed, was hypothesised to be responsible for this reduced reliance on cleaning for nutrition, and **Chapters 5** and **6** also highlight how changes in the environmental context can influence the dynamics of the interaction. Across 8 years, **Chapter 5** shows that no client species is consistently important for maintaining the occurrence of cleaning on the same reef, whilst **Chapter 6** found three biotic factors, relating to the client diversity and abundance within the community, to be consistent predictors of cleaning frequencies across years. **Chapters 7** and **8** also considered how the biotic community can influence patterns of cleaning, but at an individual level. **Chapter 7** presents a novel link between the cleaner personality traits, activity and boldness, the client species cleaned, and subsequent cleaning and posing behaviours, whilst **Chapter 8** begins to show, using a novel applied photo-identification technique, within-species differences in client behaviour at cleaning stations. Together, these seven chapters identify some factors that jointly influence the dynamic context-dependent cleaning patterns that are so frequently observed across individuals, populations and species.

HOW DID CLEANING MUTUALISMS EVOLVE AND ARE MAINTAINED?

The existence and persistence of cleaning, which is regulated by beneficial interactions between species, is an evolutionary conundrum (Herre et al. 1999; Kiers et al. 2003; Bronstein 2015). Its occurrence is to some extent, driven by parasites (**Chapter 2;** Poulin and Grutter 1996), but given that animal parasitism has evolved independently at least 60 times (Poulin and Morand 2000), it is somewhat surprising that inter- and intra-specific cleaning interactions are rare across animal phyla and families (documented in three phyla and 1.89% of animal families, **Chapter 2**). Intra-specific cleaning (commonly termed allogrooming) is a more pervasive interaction compared to inter-specific cleaning, and **Chapter 2** suggests that cleaning has evolved in some species, like fish, to exploit the lack of allogrooming amongst individuals (as also suggested by Poulin and Grutter 1996). This exploitation of clients ultimately facilitates a specialist foraging strategy for the cleaner (**Chapters 2**, **3** and **4**). Specialist strategies are maintained, and are more adaptive, in environments where food availability is more stable (Schoener 1971), which helps explain why dedicated cleaning is mostly observed in tropical aquatic environments (**Chapter 2**; Poulin and Grutter 1996; Grutter 2002; Vaughan et al. 2017): ectoparasite assemblages are larger and more diverse in these environments (Guernier et al. 2004; Bordes and Morand 2009) and parasite communities are more stable (Hawley and Altizer 2011). These specialist strategies however are only beneficial if competition for resources is low (Stephens and Krebs 1986), and thus the occurrence of the cleaners within a community will be regulated, as predicted by optimal foraging theory, by density dependent competition (Krebs 1979). Thus, only a small number of species within an environment are expected to be cleaners, which is indeed the case on coral reefs (e.g. one dedicated and three facultative cleaners observed on the Booby Reef study site in the Caribbean, **Chapter 6**; Grutter et al. 2003; Floeter et al. 2007; Sazima 2011). In **Chapter 6**, the abundance of other cleaner species on the reef did not influence cleaning patterns, providing evidence that on this study reef, cleaning still functions as a beneficial specialist foraging strategy (contrasting *Labroides dimidiatus* cleaning observed in **Chapter 4**).

Compared to other foraging methods, cleaning is not ideal (White et al. 2007); single gnathiid ectoparasites are not nutritionally very beneficial (Eckes et al. 2015, the nutritional context of soft bodied ectoparasites has not been documented however) and *Elacatinus evelynae* cleaning gobies for example had slower growth rates and high mortality compared to conspecific individuals living on and feeding off sponge (White et al. 2007). However, *Elacatinus* spp. still prefer to live on corals and clean even when sponge resources are not limiting (Whiteman and Côté 2004b), and sponge-dwellers still also occasionally clean (White et al. 2007). Combined with results from **Chapter 4** whereby the dedicated cleaner wrasse (*L. dimidiat*us) instead adopted a facultative strategy and did not completely abandon cleaning, it suggests that these dedicated species possess traits to evolve as cleaners (**Chapter 2**; Poulin and Grutter 1996), and thus they may be somewhat restricted in how much they can abandon the mutualism. In addition, the persistence of cleaning under less favourable food conditions also suggests that other benefits, such as predator protection, specialist nutritional gains (hypothesised in **Chapter 3**) and increased immunity (hypothesised for the first time in **Chapters 2** and **4**) may play an important role in driving the pervasiveness of the behaviour. Indeed, this may certainly explain why more species adopt facultative rather than dedicated cleaning strategies (Vaughan et al. 2017). The relative costs and benefits of these other factors are yet to be determined, however.

Chapter 2 novelly considers cleaning along three different continua (mutualism – parasitism, dedicated – facultative and solely inter-specific – both intra- and inter-specific), and suggests that the variable position of individuals, populations and species along these combined continua will influence the maintenance and hence occurrence of the behaviour. **Chapter 4** provided strong evidence for this idea, since under more stressed conditions, bluestreak wrasse cleaning, had at some point, shifted along all three of these continua, and thus the mutualism had, to some extent, broken down. A shift to parasitism alone, is unlikely to breakdown a mutualism (Frederickson 2017) and indeed, dedicated cleaner birds more often parasitise their clients than clean mutualistically (Weeks 2000). The mutualistic nature of cleaning is maintained by both cleaners and clients sanctioning “cheating” cleaners, and through clients soliciting further cleaning (Côté et al. 1998; Bshary and Grutter 2002a; Bshary and Schäffer 2002). Here however, **Chapters 7** and **8** suggest that this regulation may only be at a population level and individual cleaner behaviour may not positively feedback to client behaviour, and vice versa: the identities of clients cleaned versus those posed generally did not match. Ultimately this suggests a subtler form of parasitism may exist in cleaning whereby some cleaners exploit certain clients to gain increased benefits. Mutualistic cleaning is however maintained over time: some individual cleaners and/or clients may consistently deviate from mutualistic behaviour whilst others may be more mutualistic (as also shown, to some extent by Wilson et al. 2014; Found 2017), regulating population patterns. What drives these subtler between-individual differences in mutualism maintenance however is unknown.

HOW ARE CLEANING MUTUALISMS AFFECTED BY THE COMPLEX COMMUNITIES WITHIN WHICH THEY OCCUR?

The food benefits gained from cleaning will depend on the abiotic and biotic environmental context (Frederickson 2017). This context-dependency leads to highly dynamic interactions (Chamberlain et al. 2014; Hoeksema and Bruna 2015), and as shown in **Chapters 5** and **6**, cleaning and posing patterns can vary over time and between cleaning stations within the same community (supporting Sazima et al. 2000; Whiteman and Côté 2002b): **Chapters 5** and **6** provide the first demonstration of how inconsistent cleaning patterns are over longer-time scales (years). Dedicated cleaners are considered supergeneralists (Sazima et al. 2010), interacting with a vast majority of the fish community (~ up to 60% of the documented fish community in **Chapter 5**) and **Chapter 6** demonstrates, for the first time, how important the client community is in consistently regulating the occurrence of cleaning interactions across time. Different client species vary in the quantity and quality of parasitic material they host (Grutter 1994; Eckes et al. 2015), providing different rewards to the cleaner. Within and across species however, clients' ectoparasite assemblages will also be influenced by the abiotic and biotic contexts (Combes 1996; Poulin and Morand 2000) and thus the rewards each client will provide to the cleaner will also fluctuate over time and space (Grutter 1994). This explains why many theoretical potential predictors of cleaning, like client body size, are inconsistently found to influence cleaning patterns (**Chapters 3** and **6**; Grutter and Poulin 1998b; Arnal et al. 2000; Grutter et al. 2005). To ensure that cleaning remains a beneficial foraging strategy and is maintained, dedicated cleaners can increase the stability and hence their reliance on the behaviour by interacting with a diversity of clients at any one time, showing no clear preference for certain species (as shown in **Chapters 3** and **5**). Interacting with a number of similar client types will also ensure that specific nutritional benefits are still gained (e.g. **Chapters 3** and **7**). **Chapter 8** also begins to provide evidence for this at an individual level since some conspecific clients were cleaned differently to others. Ultimately having choice options will maintain the occurrence of cleaning. Indeed, reduced and/or selective choice was hypothesised to regulate the frequency of facultative cleaning in **Chapters 3** and **4**. Future work attempting to understand the dynamics and pervasiveness of cleaning would benefit greatly from investigations into the context-dependent regulation of the complex community of client parasite loads hosted within and across client species.

FUTURE DIRECTIONS - A SPATIAL APPROACH

The spatial patterning of cleaning across a reef is often overlooked, although it its well documented that clients can revisit the same individual station (**Chapter 8**; Bshary and Schäffer 2002; Soares et al. 2008b) and cleaning stations differ in their cleaning patterns (**Chapter 6**; Sazima et al. 2000). Across time, **Chapter 6** shows that no one cleaning station was consistently visited more or cleaned more than others, and although this in part will be driven by individual level behaviours of the cleaner occupying stations (as shown in **Chapter 7**), it is also likely that the clients reef use patterns will drive these temporal differences (**Chapter 8**). The spatial and temporal distributions of different reef fish will be influenced by variations in environmental conditions (Sale 1977) and home ranges of clients will ultimately restrict which stations a client can visit (e.g. sedentary damselfish with stations in their territories, Cheney and Côté 2001). Future work should consider how the clients' distribution on the reef influences temporal patterns in cleaning: do different stations cater for different client types and species (as suggested by **Chapters 7** and **8**), and are these patterns consistent across years?

The asymmetric cleaning patterns observed between cleaning stations will ultimately create an asymmetric network of interacting species (Sazima et al. 2010). Cleaning interactions can be presented as social networks (e.g. **Chapter 3: Figure 3.1,** Guimaraes et al. 2007; Sazima et al. 2010; Quimbayo et al. 2018), and social network analysis provides a useful tool for investigating how individuals interact (Croft et al. 2008). The stability of these interactions, for example, can be assessed by observing changes in the network structure when certain individuals are removed (like Pocock et al. 2012). Thus, if this method is applied to cleaning using the unique 8 year long-term data set utilised in this thesis, the robustness of cleaning to client species loss and changes could be determined. By utilising findings from **Chapter 6** on consistent versus dynamic predictors of cleaning, key client species (documented in **Chapter 5**), or cleaner personality traits (**Chapter 7**), the network structures across different cleaning stations for example could be manipulated to determine how stable the cleaning goby cleaning mutualism is over time and space. Mutualisms are not immune to breakdown (as shown in **Chapter 4**) and to fully understand how these interactions evolved and are maintained within their complex communities, it would be useful to determine at what point cleaning becomes an unstable strategy.

APPLICATIONS TO AQUACULTURE

Given the natural, health enhancing, parasite removal service provided by cleaner fish (Grutter 1999; Clague et al. 2011b; Waldie et al. 2011) it is unsurprising that the aquaculture industry has capitalised on this benefit. Cleaner fish are deployed in fish farms to biologically control the ectoparasite abundances of farmed fish (e.g. sea lice, *Lepeophtheirus salmonis* and *Caligus* spp. infecting Atlantic salmon, *Salmo salar*). This practice is most commonly observed in temperate European salmonid aquaculture (Rae 2002), where sea lice control alone, costs the industry US$480 million per annum (Costello 2009). Ironically however, there are currently no reported dedicated cleaners in temperate marine environments (Vaughan et al. 2017). Instead facultative cleaners (e.g. ballan wrasse, *Labrus bergylta*; goldsinny, *Ctenolabrus rupestris* and lumpfish, *Cyclopterus lumpus*), which are relatively large in size in comparison to their clients (e.g. *C. lumpus* mean weight per individual = 54 g, *S. salar* mean weight = 75 g, Imsland et al. 2015, **Chapter 2**), are deployed, despite these species very rarely cleaning in the wild (Potts 1973b). Although these cleaners do impact ectoparasite abundance (Rae 2002; Costello 2009; Skiftesvik et al. 2013; Imsland et al. 2014a; Leclercq et al. 2014), maintaining and enhancing the feeding efficiency of deployed cleaner fish is a major challenge for the industry. Deployed cleaners tend to be variable in their efficiency at removing parasites (Imsland et al. 2016), for example, Imsland et al. (2014a) found ingested sea lice in only ~ 30% of deployed lumpfish. It appears that deployed cleaners are opportunistic, often feeding instead on commercial feed and detritus (Deady et al. 1995; Imsland et al. 2015).

Work from this thesis ultimately suggests that current deployed cleaner species are unlikely to become more consistent cleaners of their farmed fish client species, even with interventions (e.g. artificial selection of ‘good’ *C. lumpus* individuals that show a preference for preying on sea lice, Powell et al. 2018, **Chapter 7**). As shown in **Chapter 6**, it is the diversity and abundance of different client species that maintains the occurrence of the mutualism, and as **Chapters 2** and **4** suggest, dedicated cleaners are likely to be genetically dispositioned to clean. This would explain why cleaning gobies (*Elacatinus* spp.) reduce *Neobenedenia melleni* infections of farmed fish in short term studies (e.g. ~ 8 days, dusky groupers, *Epinephelus marginatus*, see de Souza et al. 2014 and Florida red tilapia, *Oreochromis* spp., see Cowell et al. 1993). The persistence of this behaviour is however unknown, but it could be hypothesised (based on **Chapters 4** and **6**) that this interaction will break down. Interestingly, bluestreak cleaner wrasse have not yet been used in aquaculture (Vaughan et al. 2017). As shown in **Chapters 7** and **8**, the clients' behaviour is also important in regulating the occurrence of cleaning, and to date, salmonids have not been documented to pose to deployed cleaners. In the wild, salmonids are not likely to be exposed to those species which are deployed as cleaners in fish farms, and client posing can be both an innate and learnt behaviour (Losey et al. 1995; Poulin and Grutter 1996). Despite the constant abundance of salmonid food pellets within fish pens, and the lack of salmonid posing, deployed cleaners do still clean to some extent (e.g. ca. 30% of population at a sampling point, Imsland et al. 2014a). This suggests that these fish may still be gaining some form of nutritional advantage from cleaning (paralleling *in situ* results found in **Chapters 3** and **4**). In the wild, deployed cleaner species (e.g. *C. lumpus* and *L. bergylta*) consume free-living decapods and copepods (Deady and Fives 1995; Ingólfsson and Kristjánsson 2002), and thus copepod ectoparasitic sea lice consumption in fish farms, mirrors these species natural diet to some extent (**Chapter 2**). If cleaning is easily facilitated by placing together a potential cleaner with a potential client, as suggested by aquaculture, intra- and inter-specific cleaning should theoretically be more pervasive across fish, which as **Chapter 2** shows, is not the case. In tropical environments, cleaner shrimp are considered to be a sustainable option for controlling farmed fish disease (Vaughan et al. 2018b;2018a) and perhaps here instead, shrimp could also be trialled as temperate salmonid cleaners: cleaner shrimp species are documented in six families (**Chapter 2**; Vaughan et al. 2017) and all these taxa can be found in temperate marine environments (Vaughan et al. 2018b).

CONCLUSIONS

This thesis has highlighted just how complex and dynamic an iconic mutualism, the cleaner-client interaction really is. It has contributed to knowledge on how the mutualism has evolved and is maintained within the coral reef communities in which it is so ubiquitously observed. It has for the first time, identified factors which consistently regulate its dynamics, and shown just how fragile the occurrence of the interaction is to changing environments. By considering the dynamics of the mutualism at a community, species, population and individual level it highlights how all play an important interlinked role in regulating interaction patterns. Ultimately this thesis has shown the importance of choice for both cleaners and clients in maintaining the occurrence and functioning of this key, service providing, interaction: if there is little choice, and hence limited diversity in food types or quality in provided service, the mutualism is not beneficial and sustained. This may explain why cleaner fish deployed in aquaculture are poor cleaners of their single client species (see Rae 2002). The large abundance and diversity of parasite hosting reef fish occupying coral reefs (Sale 1977) ultimately drives the occurrence and maintenance of cleaning. Globally, coral reefs can be considered one of the most vulnerable environments to anthropogenic change, and already reefs have experienced large detrimental shifts in their abundance and diversity (Munday 2004; De'ath et al. 2012; Hughes et al. 2017). Findings from this thesis thus suggest that cleaning will not persist as a food acquisition strategy if reefs continue to decline. By regulating client health, cleaners can positively influence patterns of client abundance and diversity themselves (Grutter et al. 2003; Waldie et al. 2011), and thus a lack of cleaners may cause further declines to reef species diversity and abundance creating a vicious cycle.

Publication List

PUBLISHED

Dunkley K., Ellison, A., Mohammed, R.S., van Oosterhout, C. Whittey, K.E., Perkins, S.E. and Cable, J. (2019). Long-term cleaning patterns of the sharknose goby (Elacatinus evelynae). Coral Reefs. DOI: 10.1007/s00338-019-01778-9

Dunkley, K., Ioannou, C.C., Whittey, K.E., Cable, J. and Perkins, S.E. (2019). Cleaner personality and client identity have joint consequences on cleaning interaction dynamics. Behavioral Ecology. DOI: 10.1093/beheco/arz007

Dunkley, K., Cable, J. and Perkins, S.E., (2018). The selective cleaning behaviour of juvenile blue-headed wrasse (*Thalassoma bifasciatum*) in the Caribbean. Behavioural Processes, 147, 5-12.

IN PREPERATION

Dunkley, K., Cable, J. and Perkins, S. E. Context-dependency of a mutualism.

Dunkley, K., Perkins S. E. and Cable J. Role of between-individual differences in client behaviour on cleaning dynamics.

Dunkley, K., Perkins S. E. and Cable J. The cleaning-grooming continuum: from specialists to generalists.

Dunkley, K., Ward A. J. W., Perkins, S. E. and Cable, J. To clean or not to clean: cleaning mutualism breakdown in a stressed environment.

Dunkley, K., Whittey, K. E., Cable J. and Perkins S. E. Photo-identification as a tool for studying reef fish populations.

Dunkley, K., Cable J. and Perkins S. E. Role of between-individual differences in client behaviour on cleaning dynamics.

Whittey, K. E., **Dunkley, K.**, Cable, J. and Perkins, S. E. How do cleaner fish respond to changes in client abundance?

Whittey, K. E., **Dunkley, K.**, Young, G. C., Cable, J. and Perkins, S. E. The role of habitat complexity in sharknose goby (*Elacatinus evelynae*) cleaner-client interactions.

Whittey, K. E., **Dunkley, K.**, Young, G. C., Perkins, S. E. and Cable, J. Revisiting the structural complexity of cleaner stations. How do sharknose cleaner fish (*Elacatinus evelynae*) respond to changes in habitat over a 10-year period.

References

Aanen DK, Eggleton P, Rouland-Lefevre C, Guldberg-Frøslev T, Rosendahl S, Boomsma JJ. (2002). The evolution of fungus-growing termites and their mutualistic fungal symbionts. Proceedings of the National Academy of Sciences of the United States of America 99:14887-14892

Abe T, Sekiguchi K, Onishi H, Muramatsu K, Kamito T. (2012). Observations on a school of ocean sunfish and evidence for a symbiotic cleaning association with albatrosses. Marine Biology 159:1173-1176

Adam TC. (2011). High-quality habitat and facilitation ameliorate competitive effects of prior residents on new settlers. Oecologia 166:121-130

Allen JJ, Akkaynak D, Sugden AU, Hanlon RT. (2015). Adaptive body patterning, three-dimensional skin morphology and camouflage measures of the slender filefish *Monacanthus tuckeri* on a Caribbean coral reef. Biological Journal of the Linnean Society 116:377-396

Alphen JV. (1999). Evolution of colour patterns in East African cichlid fish. Journal of Evolutionary Biology 12:514-534

Altmann J. (1974). Observational Study of Behavior - Sampling Methods. Behaviour 49:227-267

Amundsen PA, Gabler HM, Staldvik FJ. (1996). A new approach to graphical analysis of feeding strategy from stomach contents data - Modification of the Costello (1990) method. Journal of Fish Biology 48:607-614

Ancillotto L, Allegrini C, Serangeli MT, Jones G, Russo D. (2014). Sociality across species: spatial proximity of newborn bats promotes heterospecific social bonding. Behavioral Ecology 26:293-299

Appeltans W, Ahyong ST, Anderson G, et al. (2012). The magnitude of global marine species diversity. Current Biology 22:2189-2202

Arnal C, Côté IM. (1998). Interactions between cleaning gobies and territorial damselfish on coral reefs. Animal Behaviour 55:1429-1442

Arnal C, Côté IM, Morand S. (2001). Why clean and be cleaned? The importance of client ectoparasites and mucus in a marine cleaning symbiosis. Behavioral Ecology and Sociobiology 51:1-7

Arnal C, Côté IM, Sasal P, Morand S. (2000). Cleaner-client interactions on a Caribbean reef: influence of correlates of parasitism. Behavioral Ecology and Sociobiology 47:353-358

Arnold EN. (1986). Mite pockets of lizards, a possible means of reducing damage by ectoparasites. Biological Journal of the Linnean Society 29:1-21

Arzoumanian Z, Holmberg J, Norman B. (2005). An astronomical pattern-matching algorithm for computer-aided identification of whale sharks *Rhincodon typus*. Journal of Applied Ecology 42:999-1011

Ashley PJ. (2007). Fish welfare: Current issues in aquaculture. Applied Animal Behaviour Science 104:199-235

Auger-Méthé M, Whitehead H. (2007). The use of natural markings in studies of long-finned pilot whales (*Globicephala melas*). Marine Mammal Science 23:77-93

Aureli F, Yates K. (2010). Distress prevention by grooming others in crested black macaques. Biology Letters 6:27-29

Ayre D, Veron J, Dufty S. (1991). The corals *Acropora palifera* and *Acropora cuneata* are genetically and ecologically distinct. Coral Reefs 10:13-18

Bachiller E, Irigoien X. (2012). Allometric relations and consequences for feeding in small pelagic fish in the Bay of Biscay. ICES Journal of Marine Science 70:232-243

Bąk B, Wilde J. (2015). Grooming behavior by worker bees of various subspecies of honey bees to remove *Varroa destructor* mites. Journal of Apicultural Research 54:207-215

Baliga VB, Mehta RS. (2015). Linking cranial morphology to prey capture kinematics in three cleaner wrasses: L*abroides dimidiatus, Larabicus quadrilineatus,* and *Thalassoma lutescens*. Journal of Morphology 276:1377-1391

Balvanera P, Pfisterer AB, Buchmann N, He JS, Nakashizuka T, Raffaelli D, Schmid B. (2006). Quantifying the evidence for biodiversity effects on ecosystem functioning and services. Ecology Letters 9:1146-1156

Barber I, Hoare D, Krause J. (2000). Effects of parasites on fish behaviour: a review and evolutionary perspective. Reviews in Fish Biology and Fisheries 10:131-165

Barbu L, Guinand C, Bergmuller R, Alvarez N, Bshary R. (2011). Cleaning wrasse species vary with respect to dependency on the mutualism and behavioural adaptations in interactions. Animal Behaviour 82:1067-1074

Bates D, Maechler M, Bolker B, Walker S. (2015). Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67:1-48

Bateson P, Laland KN. (2013). Tinbergen's four questions: an appreciation and an update. Trends in Ecology & Evolution 28:712-718

Bauer RT. (1978). Antifouling adaptations of caridean shrimps: cleaning of the antennal flagellum and general body grooming. Marine Biology 49:69-82

Bauer RT. (2004). Remarkable shrimps: adaptations and natural history of the carideans. University of Oklahoma Press

Bauer RT. (2013). Adaptive modification of appendages for grooming (cleaning; antifouling) and reproduction in the Crustacea. Functional Morphology of Crustacea 1:337-375

Becker JHA, Grutter AS. (2005). Client fish ectoparasite loads and cleaner shrimp *Urocaridella* sp c hunger levels affect cleaning behaviour. Animal Behaviour 70:991-996

Becker JHA, Curtis LM, Grutter AS. (2005). Cleaner shrimp use a to advertise cleaning rocking dance service to clients. Current Biology 15:760-764

Bell AM, Hankison SJ, Laskowski KL. (2009). The repeatability of behaviour: a meta-analysis. Animal Behaviour 77:771-783

Bellwood DR, Hughes TP, Folke C, Nystrom M. (2004). Confronting the coral reef crisis. Nature 429:827-833

Bendik NF, Morrison TA, Gluesenkamp AG, Sanders MS, O'Donnell LJ. (2013). Computer-Assisted Photo Identification Outperforms Visible Implant Elastomers in an Endangered Salamander, *Eurycea tonkawae*. PLOS ONE 8:e59424

Benzie J, Haskell A, Lehman H. (1995). Variation in the genetic composition of coral (*Pocillopora damicornis* and *Acropora palifera*) populations from different reef habitats. Marine Biology 121:731-739

Bevan PA, Gosetto I, Jenkins ER, Barnes I, Ioannou CC. (2018). Regulation between personality traits: individual social tendencies modulate whether boldness and leadership are correlated. Proceedings of the Royal Society B: Biological Sciences 285:20180829

Bever JD. (2002). Negative feedback within a mutualism: host-specific growth of mycorrhizal fungi reduces plant benefit. Proceedings of the Royal Society B: Biological Sciences 269:2595-2601

Billick I, Tonkel K. (2003). The relative importance of spatial vs. temporal variability in generating a conditional mutualism. Ecology 84:289-295

Binning SA, Shaw AK, Roche DG. (2017a). Parasites and host performance: incorporating infection into our understanding of animal movement. Integrative and Comparative Biology 57:267-280

Binning SA, Rey O, Wismer S, Triki Z, Glauser G, Soares MC, Bshary R. (2017b). Reputation management promotes strategic adjustment of service quality in cleaner wrasse. Scientific Reports 7:8425

Biro PA, Stamps JA. (2008). Are animal personality traits linked to life-history productivity? Trends in Ecology & Evolution 23:361-368

Blanchard P, Pays O, Fritz H. (2017). Ticks or lions: trading between allogrooming and vigilance in maternal care. Animal Behaviour 129:269-279

Bolger DT, Morrison TA, Vance B, Lee D, Farid H. (2012). A computer-assisted system for photographic mark–recapture analysis. Methods in Ecology and Evolution 3:813-822

Bolker BM, Brooks ME, Clark CJ, Geange SW, Poulsen JR, Stevens MH, White JS. (2009). Generalized linear mixed models: a practical guide for ecology and evolution. Trends in Ecology & Evolution 24:127-135

Bolnick DI, Amarasekare P, Araujo MS, et al. (2011). Why intraspecific trait variation matters in community ecology. Trends in Ecology & Evolution 26:183-192

Bordes F, Morand S. (2009). Parasite diversity: an overlooked metric of parasite pressures? Oikos 118:801-806

Borries C. (1992). Grooming site preferences in female langurs (*Presbytis entellus*). International Journal of Primatology 13:19-32

Breton LM, Addicott JF. (1992). Density-dependent mutualism in an aphid-ant interaction. Ecology 73:2175-2180

Bridcut EE, Giller PS. (1995). Diet variability and foraging strategies in brown trout (*Salmo trutta*): an analysis from subpopulations to individuals. Canadian Journal of Fisheries and Aquatic Sciences 52:2543-2552

Brommer JE, Class B. (2017). Personality from the perspective of behavioral ecology. In: Vonk J, Weiss A, Kuczaj SA (ed). Personality in Nonhuman Animals. Springer International Publishing, Berlin, Germany, pp73-107

Bron JE, Treasurer JW. (2009). Sea lice (Caligidae) on wrasse (Labridae) from selected British wild and salmon-farm sources. Journal of the Marine Biological Association of the United Kingdom 72:645-650

Bronstein J, Dieckmann U, Ferrière R. (2004). Coevolutionary Dynamics and the Conservation of Mutualisms. In: Ferrière R, Dieckmann U, Couvet D (ed). Evolutionary Conservation Biology. Cambridge University Press, Cambridge, UK,

Bronstein JL. (1994). Our current understanding of mutualism. Quarterly Review of Biology 69:31-51

Bronstein JL. (2015). Mutualism. Oxford University Press, Oxford, UK

Bronstein JL, Barbosa P. (2002). Multitrophic/multispecies mutualistic interactions: the role of non-mutualists in shaping and mediating mutualisms. In: Tscharntke T, Hawkins BA (ed). Multitrophic level interactions. Cambridge University Press, Cambridge, UK, pp44-65

Bronstein JL, Alarcon R, Geber M. (2006). The evolution of plant-insect mutualisms. New Phytologist 172:412-428

Brown BL, Creed RP, Skelton J, Rollins MA, Farrell KJ. (2012). The fine line between mutualism and parasitism: complex effects in a cleaning symbiosis demonstrated by multiple field experiments. Oecologia 170:199-207

Brown JH, Whitham TG, Morgan Ernest SK, Gehring CA. (2001). Complex species interactions and the dynamics of ecological systems: long-term experiments. Science 293:643-650

Bshary R. (2002a). Building up relationships in asymmetric co-operation games between the cleaner wrasse *Labroides dimidiatus* and client reef fish. Behavioral Ecology and Sociobiology 52:365-371

Bshary R. (2002b). Biting cleaner fish use altruism to deceive image-scoring client reef fish. Proceedings of the Royal Society B: Biological Sciences 269:2087-2093

Bshary R, Würth M. (2001). Cleaner fish *Labroides dimidiatus* manipulate client reef fish by providing tactile stimulation. Proceedings of the Royal Society B: Biological Sciences 268:1495-1501

Bshary R, Grutter AS. (2002a). Asymmetric cheating opportunities and partner control in a cleaner fish mutualism. Animal Behaviour 63:547-555

Bshary R, Grutter AS. (2002b). Parasite distribution on client reef fish determines cleaner fish foraging patterns. Marine Ecology Progress Series 235:217-222

Bshary R, Schäffer D. (2002). Choosy reef fish select cleaner fish that provide high-quality service. Animal Behaviour 63:557-564

Bshary R, Noë R. (2003). Biological markets: the ubiquitous influence of partner choice on the dynamics of cleaner fish-client reef fish interactions. Genetic and Cultural Evolution of Cooperation 9:167-184

Bshary R, Grutter AS. (2006). Image scoring and cooperation in a cleaner fish mutualism. Nature 441:975-978

Bshary R, Bronstein JL. (2011). A general scheme to predict partner control mechanisms in pairwise cooperative interactions between unrelated individuals. Ethology 117:271-283

Bulmer MS, Denier D, Velenovsky J, Hamilton C. (2012). A common antifungal defense strategy in *Cryptocercus* woodroaches and termites. Insectes Sociaux 59:469-478

Buray N, Mourier J, Planes S, Clua E. (2009). Underwater photo-identification of sicklefin lemon sharks, *Negaprion acutidens*, at Moorea (French Polynesia). Cybium 33:21-27

Caci G, Biscaccianti AB, Cistrone L, Bosso L, Garonna AP, Russo D. (2013). Spotting the right spot: computer-aided individual identification of the threatened cerambycid beetle *Rosalia alpina*. Journal of Insect Conservation 17:787-795

Camp EF, Nitschke MR, Rodolfo-Metalpa R, et al. (2017). Reef-building corals thrive within hot-acidified and deoxygenated waters. Scientific Reports 7:2434

Cardoso SC, Grutter AS, Paula JR, et al. (2015). Forebrain neuropeptide regulation of pair association and behavior in cooperating cleaner fish. Physiology & Behavior 145:1-7

Careau V, Thomas D, Humphries MM, Reale D. (2008). Energy metabolism and animal personality. Oikos 117:641-653

Carr MH, Anderson TW, Hixon MA. (2002). Biodiversity, population regulation, and the stability of coral-reef fish communities. Proceedings of the National Academy of Sciences 99:11241-11245

Carson R. (1962). Silent spring. Houghton Mifflin Harcourt, Boston, Massachusetts, US

Caves EM, Frank TM, Johnsen S. (2016). Spectral sensitivity, spatial resolution and temporal resolution and their implications for conspecific signalling in cleaner shrimp. Journal of Experimental Biology 219:597-608

Chamberlain SA, Bronstein JL, Rudgers JA. (2014). How context dependent are species interactions? Ecology Letters 17:881-890

Chambers SD, Sikkel PC. (2002). Diel emergence patterns of ecologically important, fish-parasitic, gnathiid isopod larvae on Caribbean coral reefs. Caribbean Journal of Science 38:37-43

Chaplin DD. (2010). Overview of the immune response. Journal of Allergy and Clinical Immunology 125:3-23

Chapuis L, Bshary R. (2009). Strategic adjustment of service quality to client identity in the cleaner shrimp, *Periclimenes longicarpus*. Animal Behaviour 78:455-459

Chapuis L, Bshary R. (2010). Signalling by the cleaner shrimp *Periclimenes longicarpus*. Animal Behaviour 79:645-647

Cheney KL. (2013). Cleaner fish coloration decreases predation risk in aggressive fangblenny mimics. Behavioral Ecology 24:1161-1165

Cheney KL, Côté IM. (2001). Are Caribbean cleaning symbioses mutualistic? Costs and benefits of visiting cleaning stations to longfin damselfish. Animal Behaviour 62:927-933

Cheney KL, Côté IM. (2003). Do ectoparasites determine cleaner fish abundance? Evidence on two spatial scales. Marine Ecology Progress Series 263:189-196

Cheney KL, Côté IM. (2005). Mutualism or parasitism? The variable outcome of cleaning symbioses. Biology Letters 1:162-165

Cheney KL, Bshary R, Grutter AS. (2008). Cleaner fish cause predators to reduce aggression toward bystanders at cleaning stations. Behavioral Ecology 19:1063-1067

Cheney KL, Grutter AS, Blomberg SP, Marshall NJ. (2009). Blue and yellow signal cleaning behavior in coral reef fishes. Current Biology 19:1283-1287

Chomicki G, Renner SS. (2017). Partner abundance controls mutualism stability and the pace of morphological change over geologic time. Proceedings of the National Academy of Sciences of the United States of America 114:3951-3956

Clague G, Newport C, Grutter A. (2011a). Intraspecific cleaning behaviour of adult cleaner wrasse, *Labroides dimidiatus* (Perciformes: Labridae). Marine Biodiversity Records 4:e56

Clague GE, Cheney KL, Goldizen AW, McCormick MI, Waldie PA, Grutter AS. (2011b). Long-term cleaner fish presence affects growth of a coral reef fish. Biology Letters 7:863-865

Clutton-Brock TH. (1989). Review lecture: mammalian mating systems. Proceedings of the Royal Society B: Biological Sciences 236:339-372

Cole AJ. (2009). Cleaning to corallivory: ontogenetic shifts in feeding ecology of tubelip wrasse. Coral Reefs 29:125-129

Combes C. (1996). Parasites, biodiversity and ecosystem stability. Biodiversity and Conservation 5:953-962

Connell JH. (1978). Diversity in tropical rain forests and coral reefs. Science 199:1302-1310

Cords M. (1995). Predator Vigilance Costs of Allogrooming in Wild Blue Monkeys. Behaviour 132:559-569

Costello MJ. (2009). The global economic cost of sea lice to the salmonid farming industry. Journal of Fish Diseases 32:115-118

Côté IM. (2000). Evolution and ecology of cleaning symbioses in the sea. Oceanography and Marine Biology 38:311-355

Côté IM, Molloy PP. (2003). Temporal variation in cleanerfish and client behaviour: does it reflect ectoparasite availability? Ethology 109:487-499

Côté IM, Soares MC. (2011). Gobies as cleaners. In: The Biology of Gobies. Science Publishers, St. Helie, pp525-551

Côté IM, Arnal C, Reynolds JD. (1998). Variation in posing behaviour among fish species visiting cleaning stations. Journal of Fish Biology 53:256-266

Cowell LE, Watanabe WO, Head WD, Grover JJ, Shenker JM. (1993). Use of tropical cleaner fish to control the ectoparasite *Neobenedenia melleni* (Monogenea: Capsalidae) on seawater-cultured Florida red tilapia. Aquaculture 113:189-200

Cremer S, Armitage SA, Schmid-Hempel P. (2007). Social immunity. Current Biology 17:R693-702

Croft DP, James R, Krause J. (2008). Exploring animal social networks. Princeton University Press

Crofton H. (1971). A quantitative approach to parasitism. Parasitology 62:179-193

Csata E, Eros K, Marko B. (2014). Effects of the ectoparasitic fungus *Rickia wasmannii* on its ant host *Myrmica scabrinodis*: changes in host mortality and behavior. Insectes Sociaux 61:247-252

Dala-Corte RB, Moschetta JB, Becker FG. (2016). Photo-identification as a technique for recognition of individual fish: a test with the freshwater armored catfish *Rineloricaria aequalicuspis* Reis & Cardoso, 2001 (Siluriformes: Loricariidae). Neotropical Ichthyology 14:e150074

Darcy GH, Maisel E, Ogden JC. (1974). Cleaning preferences of the gobies *Gobiosoma evelynae* and *G. prochilos* and the juvenile wrasse *Thalassoma bifasciatum*. Copeia:375-379

David M, Cezilly F, Giraldeau LA. (2011). Personality affects zebra finch feeding success in a producer-scrounger game. Animal Behaviour 82:61-67

Dawkins MS. (2003). Behaviour as a tool in the assessment of animal welfare. Zoology 106:383-387

de Souza RA, da Annunciação WF, Lins SM, Sanches EG, Martins ML, Tsuzuki MY. (2014). Can barber goby *Elacatinus figaro* control *Neobedenia melleni* infections on dusky grouper *Epinephelus marginatus*? Aquaculture Research 45:619-628

De'ath G, Fabricius KE, Sweatman H, Puotinen M. (2012). The 27-year decline of coral cover on the Great Barrier Reef and its causes. Proceedings of the National Academy of Sciences of the United States of America 109:17995-17999

Deady S, Fives JM. (1995). Diet of ballan wrasse, *Labrus bergylta*, and some comparisons with the diet of corkwing wrasse, *Crenilabrus melops*. Journal of the Marine Biological Association of the United Kingdom 75:651-665

Deady S, Varian SJ, Fives JM. (1995). The use of cleaner-fish to control sea lice on two Irish salmon (*Salmo salar*) farms with particular reference to wrasse behaviour in salmon cages. Aquaculture 131:73-90

Di Bitetti MS. (1997). Evidence for an important social role of allogrooming in a platyrrhine primate. Animal Behaviour 54:199-211

Dobson AP, Hudson PJ. (1986). Parasites, disease and the structure of ecological communities. Trends in Ecology & Evolution 1:11-15

Dunbar RIM. (1991). Functional-Significance of Social Grooming in Primates. Folia Primatologica 57:121-131

Eckes M, Dove S, Siebeck UE, Grutter AS. (2015). Fish mucus versus parasitic gnathiid isopods as sources of energy and sunscreens for a cleaner fish. Coral Reefs 34:823-833

EL-Ganainy AA, Sabra MMM. (2008). Age, growth, mortality and yield per recruit of the filefish *Stephanolepis diaspros* (Fraser-Brunner, 1940)(Pisces: Monacanthidae), in the Gulf of Suez, Egypt. Journal of Fisheries and Aquatic Science 3:252-260

Elliot SL, Blanford S, Thomas MB. (2002). Host-pathogen interactions in a varying environment: temperature, behavioural fever and fitness. Proceedings of the Royal Society B: Biological Sciences 269:1599-1607

Engh AL, Beehner JC, Bergman TJ, Whitten PL, Hoffmeier RR, Seyfarth RM, Cheney DL. (2006). Behavioural and hormonal responses to predation in female chacma baboons (*Papio hamadryas ursinus*). Proceedings of the Royal Society B-Biological Sciences 273:707-712

Fagan WF, Lewis MA, Auger-Methe M, et al. (2013). Spatial memory and animal movement. Ecology Letters 16:1316-1329

Fairbanks BM, Hawley DM, Alexander KA. (2014). No evidence for avoidance of visibly diseased conspecifics in the highly social banded mongoose (*Mungos mungo*). Behavioral Ecology and Sociobiology 69:371-381

Feddern HA. (1965). The spawning, growth, and general behavior of the bluehead wrasse, *Thalassoma bifasciatum* (Pisces: Labridae). Bulletin of Marine Science 15:896-941

Feder HM. (1966). Cleaning symbiosis in the marine environment. Symbiosis 1:327-380

Fefferman NH, Traniello JFA, Rosengaus RB, Calleri DV. (2006). Disease prevention and resistance in social insects: modeling the survival consequences of immunity, hygienic behavior, and colony organization. Behavioral Ecology and Sociobiology 61:565-577

Floeter SR, Vazquez DP, Grutter AS. (2007). The macroecology of marine cleaning mutualisms. Journal of Animal Ecology 76:105-111

Found R. (2017). Interactions between cleaner-birds and ungulates are personality dependent. Biology Letters 13:20170536

Francini-Filho RB, Sazima I. (2008). A comparative study of cleaning activity of two reef fishes at Fernando de Noronha Archipelago, tropical West Atlantic. Environmental Biology of Fishes 83:213-220

Francini–Filho R, Moura R, Sazima I. (2000). Cleaning by the wrasse *Thalassoma noronhanum*, with two records of predation by its grouper client *Cephalopholis fulva*. Journal of Fish Biology 56:802-809

Fraser D, Duncan IJ, Edwards SA, et al. (2013). General Principles for the welfare of animals in production systems: the underlying science and its application. The Veterinary Journal 198:19-27

Freckleton RP, Côté IM. (2003). Honesty and cheating in cleaning symbioses: evolutionarily stable strategies defined by variable pay-offs. Proceedings of the Royal Society B: Biological Sciences 270:299-305

Frederickson ME. (2013). Rethinking mutualism stability: cheaters and the evolution of sanctions. The Quarterly Review of Biology 88:269-295

Frederickson ME. (2017). Mutualisms are not on the verge of breakdown. Trends in Ecology & Evolution 32:727-734

Froese R, Pauly D. (2018). FishBase. World Wide Web electronic publication

Frost AJ, Winrow-Giffen A, Ashley PJ, Sneddon LU. (2007). Plasticity in animal personality traits: does prior experience alter the degree of boldness? Proceedings of the Royal Society B: Biological Sciences 274:333-339

Fujishima Y, Wada K. (2013). Allocleaning behavior by the sentinel crab *Macrophthalmus banzai*: a case of mutual cooperation. Journal of Ethology 31:219-221

Gans C, Baic D. (1977). Regional specialization of reptilian scale surfaces: relation of texture and biologic role. Science 195:1348-1350

García-Charton J, Pérez-Ruzafa A. (2001). Spatial pattern and the habitat structure of a Mediterranean rocky reef fish local assemblage. Marine Biology 138:917-934

Gardner TA, Cote IM, Gill JA, Grant A, Watkinson AR. (2005). Hurricanes and Caribbean coral reefs: impacts, recovery patterns, and role in long-term decline. Ecology 86:174-184

Gibson CM, Hunter MS. (2010). Extraordinarily widespread and fantastically complex: comparative biology of endosymbiotic bacterial and fungal mutualists of insects. Ecology Letters 13:223-234

Giglio VJ, Adelir-Alves J, Bertoncini AA. (2014). Using scars to photo-identify the goliath grouper, *Epinephelus itajara*. Marine Biodiversity Records 7:e108

Gill SA. (2012). Strategic use of allopreening in family-living wrens. Behavioral Ecology and Sociobiology 66:757-763

Gilmour ME, Castillo-Guerrero JA, Fleishman AB, Hernandez-Vazquez S, Young HS, Shaffer SA. (2018). Plasticity of foraging behaviors in response to diverse environmental conditions. Ecosphere 9

Gingins S, Bshary R. (2014). Pairs of cleaner fish prolong interaction duration with client reef fish by increasing service quality. Behavioral Ecology 26:350-358

Gonzalez-Teuber M, Heil M. (2009). The role of extrafloral nectar amino acids for the preferences of facultative and obligate ant mutualists. Journal of Chemical Ecology 35:459-468

Gormley AM, Slooten E, Dawson S, Barker RJ, Rayment W, du Fresne S, Bräger S. (2012). First evidence that marine protected areas can work for marine mammals. Journal of Applied Ecology 49:474-480

Gratwicke B, Speight MR. (2005). The relationship between fish species richness, abundance and habitat complexity in a range of shallow tropical marine habitats. Journal of Fish Biology 66:650-667

Groner M, Cox R, Gettinby G, Revie CW. (2013). Use of agent-based modelling to predict benefits of cleaner fish in controlling sea lice, *Lepeophtheirus salmonis*, infestations on farmed Atlantic salmon, *Salmo salar* L. Journal of Fish Diseases 36:195-208

Grutter AS. (1994). Spatial and temporal variations of the ectoparasites of seven reef fish species from Lizard Island and Heron Island, Australia. Marine Ecology Progress Series 115:21-30

Grutter AS. (1995a). Relationship between cleaning rates and ectoparasite loads in coral-reef fishes. Marine Ecology Progress Series 118:51-58

Grutter AS. (1995b). Comparison of methods for sampling ectoparasites from coral reef fishes. Marine and Freshwater Research 46:897-903

Grutter AS. (1996a). Parasite removal rates by the cleaner wrasse *Labroides dimidiatus*. Marine Ecology Progress Series 130:61-70

Grutter AS. (1996b). Experimental demonstration of no effect by the cleaner wrasse *Labroides dimidiatus* (Cuvier and Valenciennes) on the host fish *Pomacentrus moluccensis* (Bleeker). Journal of Experimental Marine Biology and Ecology 196:285-298

Grutter AS. (1999). Cleaner fish really do clean. Nature 398:672-673

Grutter AS. (2001). Parasite infection rather than tactile stimulation is the proximate cause of cleaning behaviour in reef fish. Proceedings of the Royal Society B: Biological Sciences 268:1361-1365

Grutter AS. (2002). Cleaning symbioses from the parasites' perspective. Parasitology 124 Suppl:S65-81

Grutter AS. (2003). Feeding ecology of the fish ectoparasite *Gnathia* sp. (Crustacea : Isopoda) from the Great Barrier Reef, and its implications for fish cleaning behaviour. Marine Ecology Progress Series 259:295-302

Grutter AS, Poulin R. (1998a). Intraspecific and interspecific relationships between host size and the abundance of parasitic larval gnathiid isopods on coral reef fishes. Marine Ecology Progress Series 164:263-271

Grutter AS, Poulin R. (1998b). Cleaning of coral reef fishes by the wrasse *Labroides dimidiatus*: influence of client body size and phylogeny. Copeia 1998:120-127

Grutter AS, Bshary R. (2003). Cleaner wrasse prefer client mucus: support for partner control mechanisms in cleaning interactions. Proceedings of the Royal Society B: Biological Sciences 270 Suppl 2:S242-244

Grutter AS, Bshary R. (2004). Cleaner fish, *Labroides dimidiatus*, diet preferences for different types of mucus and parasitic gnathiid isopods. Animal Behaviour 68:583-588

Grutter AS, McCallum H, Lester RJG. (2002). Optimising cleaning behaviour: minimising the costs and maximising ectoparasite removal. Marine Ecology Progress Series 234:257-264

Grutter AS, Murphy JM, Choat JH. (2003). Cleaner fish drives local fish diversity on coral reefs. Current Biology 13:64-67

Grutter AS, Glover S, Bshary R. (2005). Does client size affect cleaner fish choice of client? An empirical test using client fish models. Journal of Fish Biology 66:1748-1752

Grutter AS, Rumney JG, Sinclair-Taylor T, Waldie P, Franklin CE. (2011). Fish mucous cocoons: the 'mosquito nets' of the sea. Biology Letters 7:292-294

Guernier V, Hochberg ME, Guegan JF. (2004). Ecology drives the worldwide distribution of human diseases. PLoS Biology 2:e141

Guimaraes PR, Jr., Sazima C, dos Reis SF, Sazima I. (2007). The nested structure of marine cleaning symbiosis: is it like flowers and bees? Biology Letters 3:51-54

Gutmann AK, Spinka M, Winckler C. (2015). Long-term familiarity creates preferred social partners in dairy cows. Applied Animal Behaviour Science 169:1-8

Hamilton WD. (1963). The evolution of altruistic behavior. American Naturalist 97:354-356

Hansen MJ, O'Leary PM, Ward AJ. (2017). Interactions between *Plagiotremus* spp., *Labroides dimidiatus* and their clients: evidence for behavioural niche partitioning. Journal of Fish Biology 90:424-434

Harding JA, Almany GR, Houck LD, Hixon MA. (2003). Experimental analysis of monogamy in the Caribbean cleaner goby, *Gobiosoma evelynae*. Animal Behaviour 65:865-874

Harrison XA, Donaldson L, Correa-Cano ME, et al. (2018). A brief introduction to mixed effects modelling and multi-model inference in ecology. PeerJ 6:e4794

Hart BL. (1990). Behavioral adaptations to pathogens and parasites: five strategies. Neuroscience and Biobehavioral Reviews 14:273-294

Hart BL, Hart LA. (1992). Reciprocal Allogrooming in Impala, *Aepyceros melampus*. Animal Behaviour 44:1073-1083

Hawley DM, Altizer SM. (2011). Disease ecology meets ecological immunology: understanding the links between organismal immunity and infection dynamics in natural populations. Functional Ecology 25:48-60

Hay ME. (1997). The ecology and evolution of seaweed-herbivore interactions on coral reefs. Coral Reefs 16:S67-S76

Heinze G, Wallisch C, Dunkler D. (2018). Variable selection - A review and recommendations for the practicing statistician. Biometrical Journal 60:431-449

Hemelrijk CK, Ek A. (1991). Reciprocity and interchange of grooming and support in captive chimpanzees. Animal Behaviour 41:923-935

Henazi SP, Barrett L. (1999). The value of grooming to female primates. Primates 40:47-59

Herre EA, Knowlton N, Mueller UG, Rehner SA. (1999). The evolution of mutualisms: exploring the paths between conflict and cooperation. Trends in Ecology & Evolution 14:49-53

Herrera CM. (1988). Variation in mutualisms: the spatiotemporal mosaic of a pollinator assemblage. Biological Journal of the Linnean Society 35:95-125

Heupel MR, Simpfendorfer CA. (2015). Long-term movement patterns of a coral reef predator. Coral Reefs 34:679-691

Hobson ES. (1971). Cleaning Symbiosis among California Inshore Fishes. Fishery Bulletin of the National Oceanic and Atmospheric Administration 69:491-&

Hobson ES. (1976). The rock wrasse, *Halichoeres semicinctus*, as a cleaner fish. California Fish and Game 62:73-78

Hoegh-Guldberg O, Mumby PJ, Hooten AJ, et al. (2007). Coral reefs under rapid climate change and ocean acidification. Science 318:1737-1742

Hoeksema J, Bruna E. (2015). Context-dependent outcomes of mutualistic interactions. In: Mutualism. Oxford University Press Oxford, UK, pp181-202

Hoeksema JD, Bruna EM. (2000). Pursuing the big questions about interspecific mutualism: a review of theoretical approaches. Oecologia 125:321-330

Holland JN, DeAngelis DL. (2010). A consumer-resource approach to the density-dependent population dynamics of mutualism. Ecology 91:1286-1295

Holland JN, Ness JH, Boyle A, Bronstein JL. (2005). Mutualisms as consumer-resource interactions. In: Barbosa P, Castellanos I (ed). Ecology of Predator–Prey Interactions. Oxford University Press, New York, USA, pp17-33

Hothorn T, Bretz F, Westfall P. (2008). Simultaneous inference in general parametric models. Biometrical Journal 50:346-363

Hothorn T, Hornik K, Van de Wiel MA, Zeileis A. (2006). A Lego system for conditional inference. American Statistician 60:257-263

Hourigan TF, Stanton FG, Motta PJ, Kelley CD, Carlson B. (1989). The feeding ecology of three species of Caribbean angelfishes (family Pomacanthidae). Environmental Biology of Fishes 24:105-116

Houslay TM, Vierbuchen M, Grimmer AJ, Young AJ, Wilson AJ. (2018). Testing the stability of behavioural coping style across stress contexts in the Trinidadian guppy. Functional Ecology 32:424-438

Houston AI, McNamara JM. (1999). Models of adaptive behaviour: an approach based on state. Cambridge University Press, Cambridge, UK

Huebner LK, Chadwick NE. (2012). Reef fishes use sea anemones as visual cues for cleaning interactions with shrimp. Journal of Experimental Marine Biology and Ecology 416:237-242

Hughes TP, Bellwood DR, Connolly SR. (2002). Biodiversity hotspots, centres of endemicity, and the conservation of coral reefs. Ecology Letters 5:775-784

Hughes TP, Kerry JT, Álvarez-Noriega M, et al. (2017). Global warming and recurrent mass bleaching of corals. Nature 543:373

Humann P, Deloach N. (2014). Reef Fish Identification. New World Publications Jacksonville, USA

Hunt S, Cuthill IC, Bennett AT, Church SC, Partridge JC. (2001). Is the ultraviolet waveband a special communication channel in avian mate choice? Journal of Experimental Biology 204:2499-2507

Imsland AK, Reynolds P, Eliassen G, Hangstad TA, Foss A, Vikingstad E, Elvegård TA. (2014a). The use of lumpfish (*Cyclopterus lumpus* L.) to control sea lice (*Lepeophtheirus salmonis* Krøyer) infestations in intensively farmed Atlantic salmon (*Salmo salar* L.). Aquaculture 424:18-23

Imsland AK, Reynolds P, Eliassen G, et al. (2014b). Notes on the behaviour of lumpfish in sea pens with and without Atlantic salmon present. Journal of Ethology 32:117-122

Imsland AK, Reynolds P, Eliassen G, et al. (2015). Feeding preferences of lumpfish (*Cyclopterus lumpus* L.) maintained in open net-pens with Atlantic salmon (*Salmo salar* L.). Aquaculture 436:47-51

Imsland AK, Reynolds P, Eliassen G, et al. (2016). Is cleaning behaviour in lumpfish (*Cyclopterus lumpus*) parentally controlled? Aquaculture 459:156-165

Ingólfsson A, Kristjánsson BK. (2002). Diet of juvenile lumpsucker *Cyclopterus lumpus* (Cyclopteridae) in floating seaweed: effects of ontogeny and prey availability. Copeia 2002:472-476

Ioannou CC, Dall SR. (2016). Individuals that are consistent in risk-taking benefit during collective foraging. Scientific Reports 6:33991

Ioannou CC, Payne M, Krause J. (2008). Ecological consequences of the bold-shy continuum: the effect of predator boldness on prey risk. Oecologia 157:177-182

Irwin RE, Bronstein JL, Manson JS, Richardson L. (2010). Nectar robbing: ecological and evolutionary perspectives. Annual Review of Ecology, Evolution and Systematics 41:271-292

Itzkowitz M. (1979). The feeding strategies of a facultative cleanerfish, *Thalassoma bifasciatum* (Pisces: Labridae). Journal of Zoology 187:403-413

Jacobs GH. (2009). Evolution of colour vision in mammals. Philosophical Transactions of the Royal Society B: Biological Sciences 364:2957-2967

James J, Cable J, Richardson G, Davidson KE, Mackie ASY. (2015). Two alien species of *Branchiobdellida* (Annelida: Clitellata) new to the British Isles: a morphological and molecular study. Aquatic Invasions 10:371-383

Johnson WS, Ruben P. (1988). Cleaning behavior of *Bodianus rufus*, *Thalassoma bifasciatum*, *Gobiosoma evelynae*, and *Periclimenes pedersoni* along a depth gradient at Salt River Submarine-Canyon, St-Croix. Environmental Biology of Fishes 23:225-232

Jones B, Nishiguchi M. (2004). Counterillumination in the hawaiian bobtail squid, *Euprymna scolopes* Berry (Mollusca: Cephalopoda). Marine Biology 144:1151-1155

Jones CM, Grutter AS, Cribb TH. (2004). Cleaner fish become hosts: a novel form of parasite transmission. Coral Reefs 23:521-529

Karenina K, Giljov A, Baranov V, Osipova L, Krasnova V, Malashichev Y. (2010). Visual laterality of calf-mother interactions in wild whales. PLOS ONE 5:e13787

Karlsson O, Hiby L, Lundberg T, Jussi M, Jussi I, Helander B. (2005). Photo-identification, site fidelity, and movement of female gray seals (*Halichoerus grypus*) between haul-outs in the Baltic Sea. Ambio 34:628-634

Karplus I. (2014). Symbiosis in fishes: the biology of interspecific partnerships. John Wiley & Sons

Kernohan BJ, Gitzen RA, Millspaugh JJ. (2001). Analysis of animal space use and movements. In: Radio tracking and animal populations. Elsevier, pp125-166

Kiers ET, Rousseau RA, West SA, Denison RF. (2003). Host sanctions and the legume–rhizobium mutualism. Nature 425:78

Kinsey D. (1978). Productivity and calcification estimates using slack-water periods and field enclosures. In: Stoddart DR, Johannes RE (ed). Coral reefs: research methods. Unesco, pp439-468

Knouft JH, Page LM, Plewa MJ. (2003). Antimicrobial egg cleaning by the fringed darter (Perciformes: Percidae: *Etheostoma crossopterum*): implications of a novel component of parental care in fishes. Proceedings of the Royal Society B: Biological Sciences 270:2405-2411

Koenig WD. (1997). Host preferences and behaviour of oxpeckers: co-existence of similar species in a fragmented landscape. Evolutionary Ecology 11:91-104

Kolm N, Hoffman EA, Olsson J, Berglund A, Jones AG. (2005). Group stability and homing behavior but no kin group structures in a coral reef fish. Behavioral Ecology 16:521-527

Konrad M, Vyleta ML, Theis FJ, et al. (2012). Social transfer of pathogenic fungus promotes active immunisation in ant colonies. PLoS Biology 10:e1001300

Krajewski JP. (2007). Cleaning by the occasional cleaner *Diplodus argenteus* (Perciformes: Sparidae) in south Brazil: why so few client species? Journal of the Marine Biological Association of the United Kingdom 87:1013-1016

Krakauer DC. (1995). Groups confuse predators by exploiting perceptual bottlenecks - a connectionist model of the confusion effect. Behavioral Ecology and Sociobiology 36:421-429

Krawchuk MA, Koper N, Brooks RJ. (1997). Observations of a possible cleaning symbiosis between Painted Turtles, *Chrysemys picta*, and Snapping Turtles, *Chelydra serpentina*, in central Ontario. Canadian Field-Naturalist 111:315-317

Krebs JR. (1979). Foraging strategies and their social significance. In: Social behavior and communication. Springer, pp225-270

Kulbicki M, Arnal C. (1999). Cleaning of fish ectoparasites by a palaemonidae shrimp on soft bottoms in New Caledonia. Cybium 23:101-104

Kutsukake N, Clutton-Brock TH. (2006). Social functions of allogrooming in cooperatively breeding meerkats. Animal Behaviour 72:1059-1068

Laister S, Stockinger B, Regner AM, Zenger K, Knierim U, Winckler C. (2011). Social licking in dairy cattle - Effects on heart rate in performers and receivers. Applied Animal Behaviour Science 130:81-90

Landry C. (2012). Mighty Mutualisms: the nature of plant-pollinator interactions. Nature Education Knowledge 3:37

Leclercq E, Davie A, Migaud H. (2014). Delousing efficiency of farmed ballan wrasse (*Labrus bergylta*) against *Lepeophtheirus salmonis* infecting Atlantic salmon (*Salmo salar*) post-smolts. Pest Management Science 70:1274-1282

Lee KA. (2006). Linking immune defenses and life history at the levels of the individual and the species. Integrative and Comparative Biology 46:1000-1015

Leigh EG, Jr. (2010). The evolution of mutualism. Journal of Evolutionary Biology 23:2507-2528

Lenke R. (1988). Hormonal-control of sleep-appetitive behavior and diurnal activity rhythms in the cleaner wrasse *Labroides dimidiatus* (Labridae, Teleostei). Behavioural Brain Research 27:73-85

Leonard AS, Dornhaus A, Papaj DR. (2011). Forget-me-not: complex floral displays, inter-signal interactions, and pollinator cognition. Current Zoology 57:215-224

Lester RJ, McVinish R. (2016). Does moving up a food chain increase aggregation in parasites? Journal of the Royal Society Interface 13:20160102

Lettieri L, Streelman JT. (2010). Colourful stripes send mixed messages to safe and risky partners in a diffuse cleaning mutualism. Journal of Evolutionary Biology 23:2289-2299

Leung TLF, Poulin R. (2008). Parasitism, commensalism, and mutualism: exploring the many shades of symbioses. Vie et Milieu 58:107-115

Levy K, Lerner A, Shashar N. (2014). Mate choice and body pattern variations in the Crown Butterfly fish *Chaetodon paucifasciatus* (Chaetodontidae). Biology Open 3:1245-1251

Lewis S, Roberts G, Harris MP, Prigmore C, Wanless S. (2007). Fitness increases with partner and neighbour allopreening. Biology Letters 3:386-389

Limbaugh C, Pederson H, Chace Jr FA. (1961). Shrimps that clean fishes. Bulletin of Marine Science 11:237-257

Liu L, Li G, Sun P, Lei C, Huang Q. (2015). Experimental verification and molecular basis of active immunization against fungal pathogens in termites. Scientific Reports 5:15106

Losey GS. (1979). Fish cleaning symbiosis: proximate causes of host behaviour. Animal Behaviour 27:669-685

Losey GS, Jr. (1987). Cleaning symbiosis. Symbiosis 4:229-256

Losey GS, Jr, Mahon JL, Danilowicz BS. (1995). Innate recognition by host fish of their cleaning symbiont. Ethology 100:277-283

Lu Y, Yu Q, Zamzow JP, et al. (2000). Detection of green turtle herpesviral sequence in saddleback wrasse *Thalassoma duperrey*: a possible mode of transmission of green turtle fibropapilloma. Journal of Aquatic Animal Health 12:58-63

Ludington CA. (1979). Tidal Modifications and Associated Circulation in a Platform Reef Lagoon. Marine and Freshwater Research 30:425-430

Magnhagen C, Wacker S, Forsgren E, Myhre LC, Espy E, Amundsen T. (2014). Context consistency and seasonal variation in boldness of male two-spotted gobies. PLOS ONE 9:e93354

Makecha R, Fad O, Kuczaj I, Stan A. (2012). The role of touch in the social interactions of Asian elephants (*Elephas maximus*). International Journal of Comparative Psychology 25

Malcolm HA, Gladstone W, Lindfield S, Wraith J, Lynch TP. (2007). Spatial and temporal variation in reef fish assemblages of marine parks in New South Wales, Australia - baited video observations. Marine Ecology Progress Series 350:277-290

Mallela J, Parkinson R, Day O. (2010). An assessment of coral reefs in Tobago. Caribbean Journal of Science 46:83-87

Mancera-Rodríguez NJ, Castro-Hernández JJ. (2004). Age and growth of *Stephanolepis hispidus* (Linnaeus, 1766)(Pisces: Monacanthidae), in the Canary Islands area. Fisheries Research 66:381-386

Mann J, Stanton MA, Patterson EM, Bienenstock EJ, Singh LO. (2012). Social networks reveal cultural behaviour in tool-using [corrected] dolphins. Nature Communications 3:980

Margulis L. (1993). Symbiosis in cell evolution. W. H. Freeman, New York.

Marques JM, Olsson IA. (2007). The effect of preweaning and postweaning housing on the behaviour of the laboratory mouse (*Mus musculus*). Laboratory Animals 41:92-102

Marshall AD, Dudgeon CL, Bennett MB. (2011). Size and structure of a photographically identified population of manta rays *Manta alfredi* in southern Mozambique. Marine Biology 158:1111-1124

Maruska KP, Peyton KA. (2007). Interspecific spawning between a recent immigrant and an endemic damselfish (Pisces : Pomacentridae) in the Hawaiian Islands. Pacific Science 61:211-221

Mathot KJ, Wright J, Kempenaers B, Dingemanse N. (2012). Adaptive strategies for managing uncertainty may explain personality-related differences in behavioural plasticity. Oikos 121:1009-1020

Matsuno K, Urabe M. (1999). Male-female interactions of sika deer (*Cervus nippon*) in Nara Park through allogrooming during breeding and rutting seasons. Journal of Ethology 17:41-49

McAuliffe K, Thornton A. (2015). The psychology of cooperation in animals: an ecological approach. Journal of Zoology 295:23-35

McDonald ND, Rands SA, Hill F, Elder C, Ioannou CC. (2016). Consensus and experience trump leadership, suppressing individual personality during social foraging. Science Advances 2:e1600892

Mesterton-Gibbons M, Childress MJ. (1996). Constraints on reciprocity for non-sessile organisms. Bulletin of Mathematical Biology 58:861-875

Michael SW. (1998). Reef Fishes: A Guide to Their Identification, Behaviour, and Captive Care. Vol. 1. Microcosm Ltd, Shelburne

Michiels NK, Anthes N, Hart NS, et al. (2008). Red fluorescence in reef fish: a novel signalling mechanism? BMC Ecology 8:16

Mikheev VN, Pasternak AF, Taskinen J, Valtonen TE. (2013). Grouping facilitates avoidance of parasites by fish. Parasites & Vectors 6:301

Milinski M. (1984). A predator's costs of overcoming the confusion-effect of swarming prey. Animal Behaviour 32:1157-1162

Mills SC, Côté IM. (2010). Crime and punishment in a roaming cleanerfish. Proceedings of the Royal Society B: Biological Sciences:rspb20100941

Mohammed RS, Reynolds M, James J, et al. (2016). Getting into hot water: sick guppies frequent warmer thermal conditions. Oecologia 181:911-917

Mooring MS. (1989). Ontogeny of allogrooming in mule deer (*Odocoileus hemionus*). Journal of Mammalogy 70:434-437

Mooring MS, Mundy P. (1996). Factors influencing host selection by yellow-billed oxpeckers at Matobo National Park, Zimbabwe. African Journal of Ecology 34:177-188

Mooring MS, Blumstein DT, Stoner CJ. (2004). The evolution of parasite-defence grooming in ungulates. Biological Journal of the Linnean Society 81:17-37

Mooring MS, Benjamin JE, Harte CR, Herzog NB. (2000). Testing the interspecific body size principle in ungulates: the smaller they come, the harder they groom. Animal Behaviour 60:35-45

Mora C, Tittensor DP, Adl S, Simpson AG, Worm B. (2011). How many species are there on Earth and in the ocean? PLoS Biology 9:e1001127

Moran NP, Mossop KD, Thompson RM, Wong BBM. (2016). Boldness in extreme environments: temperament divergence in a desert-dwelling fish. Animal Behaviour 122:125-133

Muir PR, Marshall PA, Abdulla A, Aguirre JD. (2017). Species identity and depth predict bleaching severity in reef-building corals: shall the deep inherit the reef? Proceedings of the Royal Society B: Biological Sciences 284:20171551

Munday PL. (2004). Habitat loss, resource specialization, and extinction on coral reefs. Global Change Biology 10:1642-1647

Murray DL, Fuller MR. (2000). A critical review of the effects of marking on the biology of vertebrates. Research techniques in animal ecology: controversies and consequences:15-64

Muscatine L, Porter JW. (1977). Reef corals: mutualistic symbioses adapted to nutrient-poor environments. Bioscience 27:454-460

Nagelkerken I, Van der Velde G, Wartenbergh S, Nugues M, Pratchett M. (2009). Cryptic dietary components reduce dietary overlap among sympatric butterflyfishes (Chaetodontidae). Journal of Fish Biology 75:1123-1143

Narvaez P, Furtado M, Neto AI, Moniz I, Azevedo JM, Soares MC. (2015). Temperate facultative cleaner wrasses selectively remove ectoparasites from their client-fish in the Azores. Marine Ecology Progress Series 540:217-226

Nash KL, Graham NAJ, Jennings S, Wilson SK, Bellwood DR, Angeler D. (2016). Herbivore cross-scale redundancy supports response diversity and promotes coral reef resilience. Journal of Applied Ecology 53:646-655

Nelson H, Geher G. (2007). Mutual grooming in human dyadic relationships: An ethological perspective. Current Psychology 26:121-140

Newton-Fisher NE, Lee PC. (2011). Grooming reciprocity in wild male chimpanzees. Animal Behaviour 81:439-446

Noë R. (2001). Biological markets: partner choice as the driving force behind the evolution of mutualisms. In: Noë R, Hammerstein P (ed). Economics in nature: Social dilemmas, mate choice and biological markets. Cambridge University Press, Cambridge, UK, pp93-118

Noë R, Hammerstein P. (1994). Biological markets: supply and demand determine the effect of partner choice in cooperation, mutualism and mating. Behavioral Ecology and Sociobiology 35:1-11

Nolet BA. (2002). Efficiency as a foraging currency in animals attaining a gain below the energetic ceiling. Behavioral Ecology 13:571-574

Oates J, Manica A, Bshary R. (2010). Roving and service quality in the cleaner wrasse *Labroides bicolor*. Ethology 116:309-315

Oksanen J, Blanchet R, Kindt P, et al. (2018). vegan: Community Ecology Package. R package version 2.5-2. https://CRAN.R-project.org/package=vegan (Last accessed: 23/10/18).

Okuno M, Tsuji K, Sato H, Fujisaki K. (2012). Plasticity of grooming behavior against entomopathogenic fungus *Metarhizium anisopliae* in the ant *Lasius japonicus*. Journal of Ethology 30:23-27

Oliver T, Palumbi S. (2011). Do fluctuating temperature environments elevate coral thermal tolerance? Coral Reefs 30:429-440

Ollerton J, Erenler H, Edwards M, Crockett R. (2014). Extinctions of aculeate pollinators in Britain and the role of large-scale agricultural changes. Science 346:1360-1362

Pagel M, Bodmer W. (2003). A naked ape would have fewer parasites. Proceedings of the Royal Society B-Biological Sciences 270:S117-S119

Palmer T, Pringle E, Stier A, Holt R. (2015). Mutualism in a community context. In: Bronstein J (ed). Mutualism. Oxford University Press, Oxford, UK, pp159–180

Palmer TM, Stanton ML, Young TP, Goheen JR, Pringle RM, Karban R. (2008). Breakdown of an ant-plant mutualism follows the loss of large herbivores from an African savanna. Science 319:192-195

Partida-Martinez LP, Monajembashi S, Greulich K-O, Hertweck C. (2007). Endosymbiont-dependent host reproduction maintains bacterial-fungal mutualism. Current Biology 17:773-777

Paterson WD, Redman P, Hiby LA, Moss SE, Hall AJ, Pomeroy P. (2013). Pup to adult photo-ID: evidence of pelage stability in gray seals. Marine Mammal Science 29:E537-E541

Patterson JE, Ruckstuhl KE. (2013). Parasite infection and host group size: a meta-analytical review. Parasitology 140:803-813

Pellmyr O, Leebens-Mack J, Huth CJ. (1996). Non-mutualistic yucca moths and their evolutionary consequences. Nature 380:155

Pennycuick C. (1978). Identification using natural markings. In: Animal Marking. Springer, pp147-159

Petanidou T, Kallimanis AS, Tzanopoulos J, Sgardelis SP, Pantis JD. (2008). Long-term observation of a pollination network: fluctuation in species and interactions, relative invariance of network structure and implications for estimates of specialization. Ecology Letters 11:564-575

Pocock MJ, Evans DM, Memmott J. (2012). The robustness and restoration of a network of ecological networks. Science 335:973-977

Potts GW. (1973a). The ethology of *Labroides dimidiatus* (cuv. & val.) (Labridae, Pisces) on Aldabra. Animal Behaviour 21:250-291

Potts GW. (1973b). Cleaning symbiosis among British fish with special reference to *Crenilabrus melops* (Labridae). Journal of the Marine Biological Association of the United Kingdom 53:1-&

Poulin R, Fitzgerald GJ. (1989). Shoaling as an anti-ectoparasite mechanism in juvenile sticklebacks (*Gasterosteus* spp.). Behavioral Ecology and Sociobiology 24:251-255

Poulin R, Vickery WL. (1995). Cleaning symbiosis as an evolutionary game: To cheat or not to cheat? Journal of Theoretical Biology 175:63-70

Poulin R, Grutter AS. (1996). Cleaning symbioses: proximate and adaptive explanations. Bioscience 46:512-517

Poulin R, Rohde K. (1997). Comparing the richness of metazoan ectoparasite communities of marine fishes: controlling for host phylogeny. Oecologia 110:278-283

Poulin R, Morand S. (2000). The diversity of parasites. The Quarterly Review of Biology 75:277-293

Powell A, Treasurer JW, Pooley CL, Keay AJ, Lloyd R, Imsland AK, Garcia de Leaniz C. (2018). Use of lumpfish for sea-lice control in salmon farming: challenges and opportunities. Reviews in Aquaculture 10:683-702

Price PW. (1980). Evolutionary biology of parasites. Princeton University Press

Prieto AA, Ryan MJ. (1978). Some observations of the social behavior of the Arizona chuckwalla, *Sauromalus obesus tumidus* (Reptilia, Lacertilia, Iguanidae). Journal of Herpetology:327-336

Pruitt JN, Stachowicz JJ, Sih A. (2012). Behavioral types of predator and prey jointly determine prey survival: potential implications for the maintenance of within-species behavioral variation. American Naturalist 179:217-227

Pruitt JN, Keiser CN, Banka BT, Liedle JS, Brooks AJ, Schmitt RJ, Holbrook SJ. (2018). Collective aggressiveness of an ecosystem engineer is associated with coral recovery. Behavioral Ecology 29:1216-1224

Puce S, Cerrano C, Di Camillo CG, Bavestrello G. (2008). Hydroidomedusae (Cnidaria: Hydrozoa) symbiotic radiation. Journal of the Marine Biological Association of the United Kingdom 88:1715-1721

Puebla O, Bermingham E, Guichard F, Whiteman E. (2007). Colour pattern as a single trait driving speciation in *Hypoplectrus* coral reef fishes? Proceedings of the Royal Society B: Biological Sciences 274:1265-1271

Quimbayo J, Floeter S, Noguchi R, et al. (2012). Cleaning mutualism in Santa Luzia (Cape Verde Archipelago) and São Tomé Islands, Tropical Eastern Atlantic. Marine Biodiversity Records 5

Quimbayo JP, Cantor M, Dias MS, Grutter AS, Gingins S, Becker JHA, Floeter SR. (2018). The global structure of marine cleaning mutualistic networks. Global Ecology and Biogeography 27:1238-1250

Quimbayo JP, Nunes LT, Ozekoski R, et al. (2017). Cleaning interactions at the only atoll in the South Atlantic. Environmental Biology of Fishes 100:865-875

R Core Team. (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria URL https://www.R-project.org/. (Last accessed: 23/10/18)

Radford AN. (2012). Post-allogrooming reductions in self-directed behaviour are affected by role and status in the green woodhoopoe. Biology Letters 8:24-27

Radford AN, Du Plessis MA. (2006). Dual function of allopreening in the cooperatively breeding green woodhoopoe, *Phoeniculus purpureus*. Behavioral Ecology and Sociobiology 61:221-230

Radhakrishna S, Singh M. (2002). Social behaviour of the slender loris (*Loris tardigradus lydekkerianus*). Folia Primatologica 73:181-196

Rae GH. (2002). Sea louse control in Scotland, past and present. Pest Management Science 58:515-520

Ramsaroop D. (1982). A preliminary survey of the coral reefs in Man-O-War Bay, Tobago. Living World, Journal of the Trinidad and Tobago Field Naturalists' Club

Randall JE. (1964). A revision of the filefish genera Amanses and Cantherhines. Copeia:331-361

Randall JE. (1967). Food habits of reef fishes of the West Indies. University of Miami, Insitute of Marine Science

Reaka-Kudla ML. (1997). The global biodiversity of coral reefs: a comparison with rain forests. Biodiversity II: Understanding and Protecting Our Biological Resources 2:551

Réale D, Reader SM, Sol D, McDougall PT, Dingemanse NJ. (2007). Integrating animal temperament within ecology and evolution. Biological Reviews of the Cambridge Philosophical Society 82:291-318

Reichard U, Sommer V. (1994). Grooming site preferences in wild white-handed gibbons (*Hylobates lar*). Primates 35:369-374

Reisser J, Proietti M, Kinas P, Sazima I. (2008). Photographic identification of sea turtles: method description and validation, with an estimation of tag loss. Endangered Species Research 5:73-82

Reynolds M, Arapi EA, Cable J. (2018). Parasite-mediated host behavioural modifications: *Gyrodactylus turnbulli* infected Trinidadian guppies increase contact rates with uninfected conspecifics. Parasitology 145:920-926

Richards-Zawacki CL. (2010). Thermoregulatory behaviour affects prevalence of chytrid fungal infection in a wild population of Panamanian golden frogs. Proceedings of the Royal Society B: Biological Sciences 277:519-528

Rigby RA, Stasinopoulos DM. (2005). Generalized additive models for location, scale and shape. Journal of the Royal Statistical Society Series C-Applied Statistics 54:507-544

Ritter EK, Amin RW. (2016). Mouth cleaning of lemon sharks, *Negaprion brevirostris*, by sharksuckers, *Echeneis naucrates*. Copeia 104:728-733

Ross D. (1983). Symbiotic relations. The Biology of Crustacea 7:163-212

Sachs JL, Simms EL. (2006). Pathways to mutualism breakdown. Trends in Ecology & Evolution 21:585-592

Sale PF. (1977). Maintenance of high diversity in coral-reef fish communities. American Naturalist 111:337-359

Salwiczek LH, Pretot L, Demarta L, et al. (2012). Adult cleaner wrasse outperform capuchin monkeys, chimpanzees and orangutans in a complex foraging task derived from cleaner-client reef fish cooperation. PLOS ONE 7:e49068

Sandland GJ, Minchella DJ. (2003). Costs of immune defense: an enigma wrapped in an environmental cloak? Trends in Parasitology 19:571-574

Sazima C, Grossman A, Bellini C, Sazima I. (2004). The moving gardens: reef fishes grazing, cleaning, and following green turtles in SW Atlantic. Cybium 28:47-53

Sazima C, Guimarães PR, Dos Reis SF, Sazima I. (2010). What makes a species central in a cleaning mutualism network? Oikos 119:1319-1325

Sazima I. (2011). Cleaner birds: a worldwide overview. Revista Brasileira De Ornitologia 19:32-47

Sazima I, Sazima C. (2010). Brazilian cleaner birds: update and brief reappraisal. Biota Neotropica 10:327-331

Sazima I, Moura RL, Sazima C. (1999). Cleaning activity of juvenile angelfish, *Pomacanthus paru*, on the reefs of the Abrolhos Archipelago, western South Atlantic. Environmental Biology of Fishes 56:399-407

Sazima I, Sazima C, Francini RB, Moura RL. (2000). Daily cleaning activity and diversity of clients of the barber goby, *Elacatinus figaro*, on rocky reefs in southeastern Brazil. Environmental Biology of Fishes 59:69-77

Schmiege PFP, D'Aloia CC, Buston PM. (2017). Anemonefish personalities influence the strength of mutualistic interactions with host sea anemones. Marine Biology 164:24

Schneider CA, Rasband WS, Eliceiri KW. (2012). NIH Image to ImageJ: 25 years of image analysis. Nature Methods 9:671

Schoener TW. (1971). Theory of feeding strategies. Annual Review of Ecology, Evolution and Systematics 2:369-404

Seid MA, Brown BV. (2009). A new host association of *Commoptera solenopsidis* (Diptera: Phoridae) with the ant *Pheidole dentata* (Hymenoptera: Formicidae) and behavioral observations. Florida Entomologist 92:309-313

Sellers AJ, Ruiz GM, Leung B, Torchin ME. (2015). Regional variation in parasite species richness and abundance in the introduced range of the invasive lionfish, *Pterois volitans*. PLOS ONE 10:e0131075

Siebeck UE. (2004). Communication in coral reef fish: the role of ultraviolet colour patterns in damselfish territorial behaviour. Animal Behaviour 68:273-282

Siebeck UE, Parker AN, Sprenger D, Mäthger LM, Wallis G. (2010). A species of reef fish that uses ultraviolet patterns for covert face recognition. Current Biology 20:407-410

Sih A, Cote J, Evans M, Fogarty S, Pruitt J. (2012). Ecological implications of behavioural syndromes. Ecology Letters 15:278-289

Sih A, Mathot KJ, Moiron M, Montiglio PO, Wolf M, Dingemanse NJ. (2015). Animal personality and state-behaviour feedbacks: a review and guide for empiricists. Trends in Ecology & Evolution 30:50-60

Sikkel PC, Fuller CA, Hunte W. (2000). Habitat/sex differences in time at cleaning stations and ectoparasite loads in a Caribbean reef fish. Marine Ecology Progress Series 193:191-199

Silvano RAM, Tibbetts IR, Grutter AS. (2012). Potential effects of fishing on cleaning interactions in a tropical reef. Coral Reefs 31:1193-1198

Silverman J, Kline D, Johnson L, et al. (2012). Carbon turnover rates in the One Tree Island reef: a 40-year perspective. Journal of Geophysical Research: Biogeosciences 117

Silvy NJ, Lopez RR, Peterson MJ. (2012). Techniques for marking wildlife. In: The wildlife techniques manual. pp230-257

Skiftesvik AB, Bjelland RM, Durif CM, Johansen IS, Browman HI. (2013). Delousing of Atlantic salmon (*Salmo salar*) by cultured vs. wild ballan wrasse (*Labrus bergylta*). Aquaculture 402:113-118

Skogen K, Helland H, Kaltenborn B. (2018). Concern about climate change, biodiversity loss, habitat degradation and landscape change: embedded in different packages of environmental concern? Journal for Nature Conservation

Slater K, Cameron E, Turner T, du Toit JT. (2008). The influence of oestrous swellings on the grooming behaviour of chimpanzees of the Budongo Forest, Uganda. Behaviour 145:1235-1246

Smith SE, Jakobsen I, Grønlund M, Smith FA. (2011). Roles of arbuscular mycorrhizas in plant phosphorus nutrition: interactions between pathways of phosphorus uptake in arbuscular mycorrhizal roots have important implications for understanding and manipulating plant phosphorus acquisition. Plant Physiology 156:1050-1057

Smith T, Allen J, Clapham P, et al. (1999). An ocean-basin-wide mark-recapture study of the North Atlantic humpback whale (*Megaptera novaeangliae*). Marine Mammal Science 15:1-32

Soares MC, Cardoso SC, Côté IM. (2007). Client preferences by Caribbean cleaning gobies: food, safety or something else? Behavioral Ecology and Sociobiology 61:1015-1022

Soares MC, Bshary R, Côté IM. (2008a). Does cleanerfish service quality depend on client value or choice options? Animal Behaviour 76:123-130

Soares MC, Côté IM, Cardoso SC, Bshary R. (2008b). The cleaning goby mutualism: a system without punishment, partner switching or tactile stimulation. Journal of Zoology 276:306-312

Soares MC, Bshary R, Cardoso SC, Côté IM. (2008c). Does competition for clients increase service quality in cleaning gobies? Ethology 114:625-632

Soares MC, Bshary R, Cardoso SC, Cote IM. (2008d). The meaning of jolts by fish clients of cleaning gobies. Ethology 114:209-214

Soares MC, Côté IM, Cardoso SC, Oliveira RF, Bshary R. (2010). Caribbean cleaning gobies prefer client ectoparasites over mucus. Ethology 116:1244-1248

Soares MC, Oliveira RF, Ros AF, Grutter AS, Bshary R. (2011). Tactile stimulation lowers stress in fish. Nature Communications 2:534

Speakman JR. (2005). Body size, energy metabolism and lifespan. Journal of Experimental Biology 208:1717-1730

Speed CW, Meekan MG, Bradshaw CJ. (2007). Spot the match–wildlife photo-identification using information theory. Frontiers in Zoology 4:2

Spruijt BM, van Hooff JA, Gispen WH. (1992). Ethology and neurobiology of grooming behavior. Physiological Reviews 72:825-852

Stachowicz JJ. (2001). Mutualism, facilitation, and the structure of ecological communities: positive interactions play a critical, but underappreciated, role in ecological communities by reducing physical or biotic stresses in existing habitats and by creating new habitats on which many species depend. Bioscience 51:235-246

Stephens DW, Krebs JR. (1986). Foraging theory. Princeton University Press, Princeton

Stephenson A. (2016). Harmonic Analysis of Tides Using TideHarmonics https://CRAN.R-project.org/package=TideHarmonics (Last accessed: 29/01/2019)

Stummer LE, Weller JA, Johnson ML, Cote IM. (2004). Size and stripes: how fish clients recognize cleaners. Animal Behaviour 68:145-150

Sulak KJ. (1975). Cleaning behaviour in the centrarchid fishes, *Lepomis macrochirus* and *Micropterus salmoides*. Animal Behaviour 23:331-334

Takeda K, Sato S, Sugawara K. (2000). The number of farm mates influences social and maintenance behaviours of Japanese Black cows in a communal pasture. Applied Animal Behaviour Science 67:181-192

Tebbich S, Bshary R, Grutter AS. (2002). Cleaner fish *Labroides dimidiatus* recognise familiar clients. Animal Cognition 5:139-145

Thébault E, Fontaine C. (2010). Stability of ecological communities and the architecture of mutualistic and trophic networks. Science 329:853-856

Theis FJ, Ugelvig LV, Marr C, Cremer S. (2015). Opposing effects of allogrooming on disease transmission in ant societies. Philosophical Transactions of the Royal Society B: Biological Sciences 370:20140108

Thibert-Plante X, Hendry AP. (2011). The consequences of phenotypic plasticity for ecological speciation. Journal of Evolutionary Biology 24:326-342

Thompson KP. (2010). Grooming the naked ape: do perceptions of disease and aggression vulnerability influence grooming behaviour in humans? A comparative ethological perspective. Current Psychology 29:288-296

Thomson JA, Gulick A, Heithaus MR. (2015). Intraspecific behavioral dynamics in a green turtle *Chelonia mydas* foraging aggregation. Marine Ecology Progress Series 532:243-256

Thresher R. (1983). Environmental correlates of the distribution of planktivorous fishes in the One Tree Reef Lagoon. Marine Ecology Progress Series 10:7-145

Tinbergen N. (1963). On aims and methods of ethology. Zeitschrift für Tierpsychologie 20:410-433

Toby KE, Palmer TM, Ives AR, Bruno JF, Bronstein JL. (2010). Mutualisms in a changing world: an evolutionary perspective. Ecology Letters 13:1459-1474

Tomazzoni AC, Pedo E, Hartz SM. (2005). Feeding associations between capybaras *Hydrochoerus hydrochaeris* (Linnaeus) (Mammalia, Hydrochaeridae) and birds in the Lami Biological Reserve, Porto Alegre, Rio Grande do Sul, Brazil. Revista Brasileira de Zoologia 22:712-716

Toscano BJ, Gownaris NJ, Heerhartz SM, Monaco CJ. (2016). Personality, foraging behavior and specialization: integrating behavioral and food web ecology at the individual level. Oecologia 182:55-69

Tragust S, Mitteregger B, Barone V, Konrad M, Ugelvig LV, Cremer S. (2013). Ants disinfect fungus-exposed brood by oral uptake and spread of their poison. Current Biology 23:76-82

Tranter C, Hughes W. (2015). Acid, silk and grooming: alternative strategies in social immunity in ants? Behavioral Ecology and Sociobiology 69:1687-1699

Treasurer J. (1997). Parasites of wrasse (Labridae) in inshore waters of west Scotland stocked as cleaner fish of sea lice (Caligidae) on farmed Atlantic salmon. Journal of Fish Biology 50:895-899

Trivers RL. (1971). The evolution of reciprocal altruism. The Quarterly Review of Biology 46:35-57

Vallon M, Grom C, Kalb N, Sprenger D, Anthes N, Lindstrom K, Heubel KU. (2016). You eat what you are: personality-dependent filial cannibalism in a fish with paternal care. Ecology and Evolution 6:1340-1352 van Buskirk J. (2012). Behavioural plasticity and environmental change. In: Behavioural responses to a changing world: mechanisms and consequences Oxford University Press, Oxford, pp145-158

van Tienhoven A, Den Hartog J, Reijns R, Peddemors V. (2007). A computer-aided program for pattern-matching of natural marks on the spotted raggedtooth shark *Carcharias taurus*. Journal of Applied Ecology 44:273-280

Vaughan DB, Grutter AS, Hutson KS. (2018a). Cleaner shrimp are a sustainable option to treat parasitic disease in farmed fish. Scientific reports 8:13959

Vaughan DB, Grutter AS, Hutson KS. (2018b). Cleaner shrimp remove parasite eggs on fish cages. Aquaculture Environment Interactions 10:429-436

Vaughan DB, Grutter AS, Costello MJ, Hutson KS. (2017). Cleaner fishes and shrimp diversity and a re-evaluation of cleaning symbioses. Fish and Fisheries 18:698-716

Vázquez DP, Melián CJ, Williams NM, Blüthgen N, Krasnov BR, Poulin R. (2007). Species abundance and asymmetric interaction strength in ecological networks. Oikos 116:1120-1127

Verbeek N, Butler RW, Richardson H. (1981). Interspecific allopreening solicitation in female Brewer's blackbirds. The Condor 83:179-180

Vergés A, Doropoulos C, Malcolm HA, et al. (2016). Long-term empirical evidence of ocean warming leading to tropicalization of fish communities, increased herbivory, and loss of kelp. Proceedings of the National Academy of Sciences of the United States of America 113:13791-13796

Villa SM, Goodman GB, Ruff JS, Clayton DH. (2016). Does allopreening control avian ectoparasites? Biology Letters 12:20160362

Volpato GL, Giaquinto PC, de Castilho MF, Barreto RE, de Freitas EG. (2009). Animal welfare: from concepts to reality. Oecologia Brasiliensis 13:5-15

Wabnitz C. (2003). From ocean to aquarium: the global trade in marine ornamental species. UNEP/Earthprint

Wagner EL, Roche DG, Binning SA, Wismer S, Bshary R. (2015). Temporal comparison and predictors of fish species abundance and richness on undisturbed coral reef patches. PeerJ 3:e1459

Waldie PA, Blomberg SP, Cheney KL, Goldizen AW, Grutter AS. (2011). Long-term effects of the cleaner fish *Labroides dimidiatus* on coral reef fish communities. PLOS ONE 6:e21201

Walker TN, Hughes WO. (2009). Adaptive social immunity in leaf-cutting ants. Biology Letters 5:446-448

Walsh CAJ, Pinheiro HT, Rocha LA, Goodbody-Gringley G. (2017). Cleaning service gaps in Bermuda, North Atlantic. Ecology 98:1973-1974

Ward AJW, Hart PJB. (2003). The effects of kin and familiarity on interactions between fish. Fish and Fisheries 4:348-358

Ward AJW, Hart PJB, Krause J. (2004). Assessment and assortment: how fishes use local and global cues to choose which school to go to. Proceedings of the Royal Society B-Biological Sciences 271:S328-S330

Watson GS, Green DW, Schwarzkopf L, Li X, Cribb BW, Myhra S, Watson JA. (2015). A gecko skin micro/nano structure - A low adhesion, superhydrophobic, anti-wetting, self-cleaning, biocompatible, antibacterial surface. Acta Biomaterialia 21:109-122

Webster MM, Ward AJ. (2011). Personality and social context. Biological Reviews of the Cambridge Philosophical Society 86:759-773

Weeks P. (2000). Red-billed oxpeckers: vampires or tickbirds? Behavioral Ecology 11:154-160

Werner EE. (1992). Individual behavior and higher-order species interactions. American Naturalist 140:S5-S32

Werner EE, Gilliam JF. (1984). The ontogenetic niche and species interactions in size structured populations. Annual Review of Ecology, Evolution and Systematics 15:393-425

West-Eberhard MJ. (1989). Phenotypic plasticity and the origins of diversity. Annual Review of Ecology, Evolution and Systematics 20:249-278

White JW, Grigsby CJ, Warner RR. (2007). Cleaning behavior is riskier and less profitable than an alternative strategy for a facultative cleaner fish. Coral Reefs 26:87-94

Whiteman EA, Côté IM. (2002a). Sex differences in cleaning behaviour and diet of a Caribbean cleaning goby. Journal of the Marine Biological Association of the United Kingdom 82:655-664

Whiteman EA, Côté IM. (2002b). Cleaning activity of two Caribbean cleaning gobies: intra- and interspecific comparisons. Journal of Fish Biology 60:1443-1458

Whiteman EA, Côté IM. (2004a). Dominance hierarchies in group-living cleaning gobies: causes and foraging consequences. Animal Behaviour 67:239-247

Whiteman EA, Côté IM. (2004b). Individual differences in microhabitat use in a Caribbean cleaning goby: a buffer effect in a marine species? Journal of Animal Ecology 73:831-840

Wickham H. (2017). scales: Scale Functions for Visualization. R package version 0.5.0

Williams ER, Thomson B. (2015). Improving population estimates of Glossy Black-Cockatoos (*Calyptorhynchus lathami*) using photo-identification. Emu 115:360-367

Wilson ADM, Krause J, Herbert-Read JE, Ward AJW. (2014). The personality behind cheating: behavioural types and the feeding ecology of cleaner fish. Ethology 120:904-912

Wilson DS. (1998). Adaptive individual differences within single populations. Philosophical Transactions of the Royal Society of London Series B: Biological Sciences 353:199-205

Wilson EO. (1989). Threats to Biodiversity. Scientific American 261:108-&

Wisenden BD, Goater CP, James CT. (2009). Behavioral defenses against parasites and pathogens. Fish Defenses 2:151-168

Wittenberger JF. (1985). The adaptive significance of coloniality in birds. Avian Biology 8:1-78

Wolf M, Weissing FJ. (2012). Animal personalities: consequences for ecology and evolution. Trends in Ecology & Evolution 27:452-461

Wong B, Candolin U. (2015). Behavioral responses to changing environments. Behavioral Ecology 26:665-673

Wong MYL, Beasley AL, Douglass T, Whalan S, Scott A. (2017). Some anemonefish lack personality: a comparative assessment of behavioral variation and repeatability in relation to environmental and social factors. Coral Reefs 36:1307-1316

Woolhouse ME, Haydon DT, Antia R. (2005). Emerging pathogens: the epidemiology and evolution of species jumps. Trends in Ecology & Evolution 20:238-244

Wyman RL, Walterswyman MF. (1985). Chafing in fishes - occurrence, ontogeny, function and evolution. Environmental Biology of Fishes 12:281-289

Youngbluth MJ. (1968). Aspects of the ecology and ethology of the cleaning fish, *Labroides phthirophagus* Randall Zeitschrift für Tierpsychologie 25:915-932

Zander CD, Sotje I. (2002). Seasonal and geographical differences in cleaner fish activity in the Mediterranean Sea. Helgoland marine research 55:232-241