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Coral reef degradation, fish parasite loads and cleaning behaviour: insights from a Caribbean island

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Resumo

Nos últimos anos tem-se verificado um preocupante declínio dos recifes de coral, para o qual têm contribuído maioritariamente fatores antropogénicos. Isto permite que as algas ganhem uma vantagem competitiva sobre os corais, criando um desequilíbrio no ecossistema, tal como uma proliferação excessiva de parasitas. Os parasitas são parte natural e indispensável dos ecossistemas, tendo um duplo papel, já que tanto são influenciados pelo ambiente, como influenciam os hospedeiros dos quais se alimentam. Tem-se vindo a demonstrar que alguns ectoparasitas evitam ativamente o contacto com coral vivo que, com a crescente degradação dos recifes, tem vindo a diminuir, levando a uma maior proliferação destes organismos parasitários. Por outro lado, grandes densidades de peixes aumentam a proximidade entre possíveis hospedeiros, aumentando assim a probabilidade de contacto com ectoparasitas, o que tornaria espécies gregárias mais vulneráveis a infeções. De forma a contrariar o aumento da carga parasitária, especialmente de ectoparasitas, os peixes infetados podem raspar-se contra o substrato ou visitar estações de limpeza. Nestes locais residem peixes ou camarões limpadores que se alimentam dos ectoparasitas de peixes maiores, chamados "clientes". Apesar de ainda haver alguma discussão em relação à natureza destas interações, elas são geralmente descritas como mutualistas. Assim, os ectoparasitas parecem ser uma variável fundamental para explicar as interações de limpeza, nas quais os clientes com maior carga parasitária visitam limpadores mais frequentemente e durante mais tempo. Estes clientes são, normalmente, aqueles que recebem um melhor e mais honesto serviço de limpeza. Para além de ectoparasitas, há outros tipos de vetores que podem levar os peixes a visitar estações de limpeza. Por exemplo, nas Caraíbas, os cirurgiões (*Acanthurus* spp.) têm sido reportados como uma família propensa ao aparecimento de manchas negras, normalmente associadas a infeções de parasitas dérmicos. O objetivo da presente dissertação foi investigar se em recifes mais degradados, ou com variações de densidade de peixes, a carga de ectoparasitas em donzelas (*Stegastes diencaeus*) e cirurgiões (*Acanthurus tractus*) seria mais elevada. Foi também investigado se os clientes mais infetados (quer por ectoparasitas, quer por parasitas dérmicos) investiam mais em comportamentos de limpeza, e se lhes era prestado um melhor serviço por parte dos limpadores. Em cinco recifes na ilha de Curaçao, Caraíbas, fizeram-se transectos de ponto-interceção para avaliar a degradação do recife. Cada ponto da comunidade bentónica foi categorizado em termos da percentagem de cobertura, em função da presença de coral vivo, areia e outros (englobando substrato favorável a ectoparasitas: coral morto, algas e esponjas). A densidade de peixes foi igualmente analisada com base em transectos, nos quais o número e espécie dos indivíduos encontrados foram registados. Os ectoparasitas de dez a onze indivíduos, tanto de donzelas como de cirurgiões, em cada recife,

foram obtidos através de banhos de água doce com óleo de cravinho. Já os comportamentos ditos de limpeza (frequência de raspagens, interações e poses) e medidas de qualidade de serviço (tempo de espera na estação de limpeza, tempo de interação, e *jolts*) advieram do seguimento e filmagem de nove a onze indivíduos de cada espécie nos dois recifes considerados como os mais diferentes (Carmabi e Water Factory). Desses mesmos peixes, mas apenas no caso dos cirurgiões, foi registado o número de manchas negras como medida da carga de parasitas dérmicos. As diferenças entre os cinco recifes foram exploradas através de testes ANOSIM, MDS, SIMPER e ANOVA / Kruskal-Wallis, enquanto as diferenças entre os dois recifes mais distintos e entre as duas espécies de clientes foram exploradas com testes Mann-Whitney. A existência de relações entre variáveis foi analisada com recurso a correlações de Spearman.

Os recifes amostrados, no que se refere ao respetivo estado, revelaram algumas diferenças, tanto na composição da comunidade bentónica como na da comunidade de peixes, especialmente entre os recifes de Carmabi e Water Factory. Water Factory foi claramente o recife com maior cobertura de coral vivo, mas também o recife com menor cobertura de substrato favorável à proliferação de parasitas em relação a Carmabi e Blue Bay Left. Water Factory e Carmabi foram os recifes com menor densidade global de peixes, mas considerando a densidade das espécies estudadas, apenas Carmabi revelou ter uma menor densidade de donzelas que Blue Bay Left. Entre os parasitas, foram identificados exemplares de três famílias: Gnathiidae (Crustacea, Isopoda), Caligidae (Crustacea, Copepoda) e Capsalidae (Platyhelminthes, Monogenea). Em todos os cinco recifes as donzelas mostraram diferenças nas três famílias de ectoparasitas, enquanto os cirurgiões mostraram diferenças apenas em duas das famílias, sendo a carga de caligídeos semelhante entre recifes. Em ambos os casos os capsalídeos mostraram resultados mais robustos, sendo em Carmabi encontradas as maiores cargas deste ectoparasita. Já a comparação entre espécies revelou que a carga de gnatiídeos foi mais elevada nas donzelas de Blue Bay Right, a carga de caligídeos foi sempre mais elevada nos cirurgiões exceto em Carmabi, que foi semelhante, e a carga de capsalídeos foi semelhante em todos os recifes. Diferentes correlações foram encontradas consoante a espécie considerada. Nas donzelas, as cargas de gnatiídeos e de caligídeos estavam negativamente correlacionadas com a cobertura de coral vivo e com a densidade da espécie, respetivamente. Nos cirurgiões, só houve uma correlação, negativa, entre cargas de gnatiídeos e a densidade global de peixes. Considerando apenas os recifes de Carmabi e Water Factory (como o mais e o menos degradado, respetivamente), a carga de ectoparasitas dos cirurgiões foi semelhante, e a das donzelas foi superior em Carmabi, maioritariamente devido aos capsalídeos. Já a frequência de manchas negras nos cirurgiões (não foi possível analisar a das donzelas) foi claramente superior em Carmabi. Em relação aos comportamentos de limpeza os

cirurgiões efetuaram mais raspagens no substrato em Carmabi, e mais que as donzelas em qualquer dos recifes. Em Carmabi foram também os cirurgiões os que esperaram mais tempo para ser atendidos pelos limpadores. Os restantes comportamentos, tais como as medidas de qualidade de serviço prestado pelos limpadores, foram semelhantes entre recifes e entre espécies. Finalmente, em Carmabi, o número de manchas dos cirurgiões revelou-se positivamente correlacionado com a frequência de interações cliente-limpador.

As cargas de capsalídeos, ectoparasitas com uma fase bentónica, foram superiores em Carmabi onde aparentemente havia uma pior qualidade de água e menor cobertura de coral vivo. Apenas as cargas de gnatiídeos, outro ectoparasita com fase bentónica, das donzelas foram mais elevadas em recifes com menor cobertura de coral vivo, provavelmente advindo de uma maior proximidade com substrato favorável a estes parasitas, promovido pela territorialidade destas. Os caligídeos foram encontrados sobretudo em cirurgiões, provavelmente devido ao seu comportamento gregário e móvel, utilizando mais a coluna de água na sua extensão. A densidade de peixes (de donzelas e global) mostrou ser um fator relevante, consoante a espécie (donzelas ou cirurgiões, respetivamente), na relação, negativa, com a carga de caligídeos e gnatiídeos, respetivamente. No entanto, a diferença na carga de ectoparasitas das donzelas não se revelou suficiente para uma intensificação do seu comportamento de limpeza, nem para a qualidade do serviço que lhes era prestado pelos organismos limpadores. Por outro lado, em Carmabi os cirurgiões investiram mais nos comportamentos de limpeza (raspagens e tempo de espera nas estações de limpeza) indo ao encontro da maior incidência de parasitas dérmicos (manchas negras) encontrada neste recife. Apenas no recife mais degradado a incidência de parasitas dérmicos revelou estar relacionada com um maior número de interações com limpadores. Assim, os níveis de degradação dos recifes de Curaçao parecem estar a afetar as cargas parasitárias de donzelas e cirurgiões, e essas mesmas cargas parasitárias parecem refletir-se especialmente numa intensificação do comportamento de limpeza nos cirurgiões, uma espécie aparentemente mais vulnerável. Estudos futuros deverão incluir uma abordagem mais integrativa, considerando, tanto ectoparasitas como parasitas dérmicos, a vulnerabilidade dos peixes, a frequência e qualidade dos comportamentos de limpeza, mas também medidas fisiológicas (como o nível de *stress*) e imunológicas. Assim, a informação resultante poderá ser importante para a monitorização da degradação dos recifes de coral e da saúde dos peixes que neles habitam, contribuindo para a conservação deste ecossistema.

Palavras-chave: Degradação de recifes de coral, parasitas, comportamento de limpeza, *Stegastes diencaeus*, *Acanthurus tractus*.

Abstract

Coral reefs are experiencing worrisome levels of degradation, mostly due to anthropogenic factors, leading to a disequilibrium in the ecosystem, such as an outbalanced parasite proliferation. Parasites are a natural component of the ecosystem, being influenced by the environment and in turn influencing their hosts. Conversely, higher fish density may also increase the probability of contact between parasites and possible hosts. In order to face parasitation, specifically by ectoparasites, highly infected fish may chafe against the substrate or engage in cleaning interactions more often. These so called "clients" are usually those that receive a better service by cleaners. There are also other sources of irritation that may lead fish to increase their cleaning behaviour, such may be the case of dermal parasites, visually recognized by dark blemishes on fish. The present study aimed to understand how reef degradation levels and fish density are affecting ectoparasite loads on fish, and how these ectoparasite loads, as well as dermal parasites, are affecting the clients' cleaning behaviour. Five reefs in Curaçao were sampled for benthic community, fish density, and ectoparasite load assessment of longfin damselfish (*Stegastes diencaeus*) and ocean surgeonfish (*Acanthurus tractus*). The cleaning behaviour of the mentioned client species was also analysed in the two most different reefs. Carmabi and Water Factory revealed to be the two most different reefs in terms of benthic and fish community, and deemed as the degraded and less degraded reefs, respectively. Ectoparasites retrieved consisted in species of the Gnathiidae (Crustacea, Isopoda), Caligidae (Crustacea, Copepoda) and Capsalidae (Platyhelminthes, Monogenea) families. Except for ocean surgeonfish's caligid loads, all ectoparasite families were found significantly different across the five sampled reefs, with fish from Carmabi experiencing the highest loads of capsalids. In four of the five reefs, ocean surgeonfish were more infected with caligids than longfin damselfish. Longfin damselfish's gnathiid and caligid loads correlated negatively with reef live coral cover and its density, respectively. Regarding ocean surgeonfish, only gnathiid loads correlated negatively with global fish density.

In terms of behavioural shifts, ectoparasite loads did not seem enough to produce changes in longfin damselfish cleaning behaviour. However, ocean surgeonfish appeared to be more vulnerable, as fish from Carmabi were observed to invest more in cleaning behaviour (chafing and waiting time at cleaning stations). This appeared to be due to significantly higher loads of dermal parasites in Carmabi (the degraded reef), where heavily infected fish interacted more often with cleaners. Future studies should include a more integrative approach, taking into consideration not only ectoparasite and dermal parasite loads, host vulnerability and cleaning behaviour, but also physiological (such as stress) and immunological measures as key variables to evaluate ecosystem

disequilibrium. The resulting information may be a valuable contribution to the implementation of monitoring programs and to help in reef conservation.

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1. INTRODUCTION

Coral reefs are among the most productive and diverse ecosystems, and although they cover only 0.2% of the ocean floor, they support approximately 25% of the marine life (Spalding *et al.*, 2001). Besides, coral reefs are vital to human societies through the goods and services provided, such as food, coastal protection, recreation and income, surpassing simple economic values and including nutritional, social and cultural values (Spalding *et al.*, 2001). Currently, coral reefs' intrinsic beauty, productivity, diversity and the values they represent are globally threatened.

1.1. CORAL REEF DEGRADATION

Coral reef degradation is presently a severe worldwide problem, affecting tropical and subtropical reefs. Both natural and anthropogenic pressures, such as storms, coral diseases, overexploitation, sedimentation, pollution, declining of water quality, and finally climate change, are amongst the main causes for reef degradation (Wilkinson, 2000; Pandolfi *et al.*, 2003; Bellwood *et al.*, 2004; Jackson, 2010). By the year 2000, 27% of reefs worldwide had come to a point of no recovery, and by 2030, estimates predict that 48% of the world's coral reefs may be lost (Wilkinson, 2000).

The Caribbean is one of the most strongly affected regions (McClanahan *et al.*, 1999; Pandolfi *et al.*, 2003; Bellwood *et al.*, 2004; Bruno *et al.*, 2009). Recent evidences indicate that in a 25 years interval, absolute hard coral cover has declined from 50% to 10% on reefs across the entire Caribbean basin (Gardner *et al.*, 2003), with 47% of the existing reefs (already in 1997) exhibiting no recovery (Roff and Mumby, 2012). Moreover, Caribbean reefs have lower functional group redundancy (both in fish and coral communities) (Bellwood *et al.*, 2004), which makes their resilience lower when compared to Indo-Pacific reefs. Consequently, Caribbean reefs are more vulnerable to threats and their recovery is even more difficult.

As coral reefs degrade, live coral gives way to an increase of dead coral covered with macroalgae, turf, cyanobacteria and sponges (Mumby *et al.*, 2007; Roff and Mumby, 2012; Artim and Sikkel, 2013). This is often characterized as a coral-algae phase shift (Hughes *et al.*, 2007; Mumby *et al.*, 2007). Usually, the consequences of reef degradation and algae phase shifts focus on corals or on fish, two important groups in the reef ecosystem. However, there are other reef habitants often ignored but no less important: the parasites.

1.2. PARASITES IN THE ECOSYSTEM

Although seemingly inconspicuous organisms, parasite species may comprise of up to 50% of all biodiversity (Hudson *et al.*, 2006). In tropical reefs, parasites may reach 80% of the organisms

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(Rohde, 2002), and the Caribbean, along with Australia, appear to be hotspots for parasites (Côté, 2000).

Indeed, parasites are a key element in the ecosystem. They have been found to be the ultimate missing link in food webs, dominating and capable of transforming food web designs as we know them (Hudson *et al.*, 2006; Dobson *et al.*, 2008; Lafferty *et al.*, 2008). Parasites may be used to distinguish stocks, tag fish populations and identify their source (through host specificity and environmental requirements) (Sasal *et al.*, 2007; Coile and Sikkel, 2013), and can also be used as bioindicators of anthropogenic influences. Ectoparasites are especially good indicators because, by infecting fish's body surface and gills and feeding on their blood, mucus and tissue (Rohde, 1984), they are in direct contact with the environment (Sures, 2004; Hudson *et al.*, 2006; Sasal *et al.*, 2007). However, it is important to first recognize that the sole existence of parasites does not mean that the environment is somehow degraded. Parasites are normally part of any healthy ecosystem, but drastic changes in their dynamics (parasite abundance and diversity baseline) should be used as key indicators (Landsberg *et al.*, 1998; Marcogliese, 2005; Hudson *et al.*, 2006). Moreover, parasites are more abundant in shallow waters, where the probability of any ectoparasite finding a host is higher (given the lower water volume), rather than deep waters (where they are more vulnerable to ocean currents) (Poulin and FitzGerald, 1989a; Grutter, 1998).

Ectoparasites can use both physical and chemical cues to recognize their hosts (Rohde, 1984; Buchmann and Lindenstrøm, 2002; Sikkel *et al.*, 2011). Physical cues, like water movements and visual cues, are non-specific, as they may be provided by fish movements or by resting or slow-moving fish (*i.e.* shadows) (Poulin *et al.*, 1990; Mikheev *et al.*, 2004). Conversely, chemical cues, like the olfactory ones, allow host-specific recognition (Mikheev *et al.*, 2004; Mordue (Luntz) and Birkett, 2009; Sikkel *et al.*, 2011), as they are often associated with fish mucus (Buchmann and Lindenstrøm, 2002), thus presenting different motivations for ectoparasites towards different meal quality (Christe *et al.*, 2003; Nagel and Grutter, 2007).

On the other hand, each host species has different susceptibility to ectoparasites, which is influenced by host specificity, spatial and temporal activity, host defences and host ability to start and maintain an appropriate immunological response (Coile and Sikkel, 2013). Nevertheless, ectoparasites are known to cause and facilitate diseases, either through the wounds they inflict while feeding, or through their activity as vectors (Davies and Smit, 2001; Panek, 2005; Cook *et al.*, 2015). Consequently, ectoparasites, as well as other parasites, may induce immunological and physiological changes in the host (Gorlick *et al.*, 1987; Bunkley-Williams and Williams, 1998; Barber *et al.*, 2000; Buchmann and Lindenstrøm, 2002).

Parasitic effects in the ecosystem may also be expressed though their influence on hosts' behaviour, population dynamics and fitness (Finley and Forrester, 2003; Hudson *et al.*, 2006). Finally, parasites can also mediate invasions (Tourchin *et al.*, 2002) and symbiotic interactions (*e.g.* Côté, 2000).

1.3. CLEANING INTERACTIONS

Cleaning interactions are considered as a typical example of symbiosis, and a remarkable example of interspecific interactions (Poulin and Grutter, 1996; Côté, 2000). Generally, these interactions may be described as the removal of ectoparasites, bacteria, injured tissue and other particles of a co-operating organism, known as "client", by a smaller organism, known as "cleaner" (Côté, 2000). Cleaning symbiosis has a wide taxonomic distribution, for instance, in terrestrial systems some birds may act as cleaners when they inspect the body of mammals for ectoparasites (Poulin and Grutter, 1996). However, most cleaning interactions have been confirmed in marine systems, especially in the tropics (Poulin and Grutter, 1996). In coral reefs, cleaners may be small fish or shrimp, usually identified by their conspicuous colours and evident stripes (Côté, 2000). As for clients, there is a much larger range, from sharks, manta rays, turtles, to a great variety of teleost fishes (Côté, 2000).

Cleaning symbioses are commonly used as examples of mutualism. Cleaning benefits cleaners, providing them with a food source gleaned from clients, whether they are obligate cleaners (rely virtually exclusively on ectoparasites) or facultative cleaners (rely only in a relatively small part on ectoparasites, and may only act as cleaners in part of their life history stages) (Côté, 2000). On the other hand, it has been harder to find significant benefits for clients. Generally, these benefits may be related to the removal of ectoparasites directly and/or to obtain tactile stimulation (Poulin and Grutter, 1996; Côté, 2000; Soares *et al.*, 2011). Yet, not all cleaners perform tactile stimulation, until now only some cleaners belonging to the family Labridae (mostly *Labroides* spp.) have been recorded as doing so (Barbu *et al.*, 2011).

However, the true nature of this symbiosis has been a matter of discussion for the past four decades (Côté, 2000). Actually, the cleaner may exploit the client, as it has been showed that cleaners not only remove ectoparasites from their clients, but also healthy tissue, mucus and scales (Poulin and Grutter, 1996; Côté, 2000; Bshary and Grutter, 2002; Côté and Soares, 2011). When this occurs the symbiotic interaction may shift towards commensalism or, in more extreme cases, parasitism (Poulin and Grutter, 1996; Cheney and Côté, 2005). Indeed, Cheney and Côté (2005) suggested that the outcome, even of the same client-cleaner interaction, may be variable

over space and time, being mainly dependent on ectoparasite availability. Cleaning interactions where there was higher ectoparasite availability, resulted in a greater decrease in clients' ectoparasite loads and less scales intake by cleaners. On the other hand, in places where ectoparasite availability was low, tissue and scales intake by cleaners seem to be more frequent.

1.4. Curaçao, an island in the Caribbean

Curaçao is an island located in the South Caribbean Sea, north to the coast of Venezuela. In Curaçao, water temperature varies between 26 °C and 28 °C throughout the year, usually not showing more than 2 °C variation regarding these values (Bak, 1975; Bak *et al.*, 2005). The reefs on the southwestern shore are typically affected by calm condition, while the northeast shore is affected by high wave activity due to east dominant winds (van Duyl, 1985).

Within the Caribbean region, Curaçao is where some of the best reefs can be found, turning it into one the five hotspots of species-diversity for the Caribbean area. In spite of this, Curaçao, like other places, has been subjected to an increase in coastal development, tourism overuse, pollution, runoff, eutrophication and overfishing which, together with climate change, resulted in a significant decrease in coral and fish abundance (Vermeij, 2012). Even though Curaçaoan reefs appear to have suffered a time-delayed coral mortality, relatively to most other Caribbean sites (Gardner *et al.*, 2003), reef degradation has reached worrisome levels. So much that, at the current rate of decline, it is predicted that by 2060 coral reefs will have virtually disappeared around Curaçao (Vermeij, 2012).

1.5. The species studied

The Pomacentridae (damselfish) and Acanthuridae (surgeonfish) are two of the three families (along with Scaridae family - parrotfish) with a significant role as herbivores on coral reefs, maintaining ecosystem integrity and resilience (Hughes *et al.*, 2007; Buchanan *et al.*, 2016). Indeed, herbivory may be a significant controller for algal distribution and abundance, depending on algae growing rate and herbivorous density (McClanahan *et al.*, 1999; Wild *et al.*, 2014; Hixon, 2015).

Longfin damselfish, *Stegastes diencaeus* (Jordan & Rutter, 1897) (Pomacentridae), is a reef-associated species, commonly found between 1 m and 25 m deep in the western Atlantic Ocean, including the Bahamas, the Gulf of Mexico, southern Florida, and throughout the Caribbean Sea (Humann and Deloach, 2002). Adults measure 5 - 13 cm TL (total length) and can be identified by distinctive features, such as dorsal and anal pointed fins, reaching well beyond the base of the

tail; and a thin bright blue edge on the anal fin. Additionally, they have a dark grey-brown body colour, often with a yellowish dorsal region, mostly on the head (Humann and Deloach, 2002). Longfin damselfish are considered as "farmer" herbivorous because they appear to maintain preferred algal species or other food items in their relatively small territories (*ca*. 1 m^2) (Cheney and Côté, 2001, 2003a; Ceccarelli *et al.*, 2005; Vermeij *et al.*, 2015). Indeed, this solitary damselfish defends its territory actively and pugnaciously from intruders (Humann and Deloach, 2002), usually other herbivorous and egg predators (Arnal and Côté, 1998; Cheney and Côté, 2003a).

The ocean surgeonfish, *Acanthurus bahianus* Castelnau, 1855, with a distribution in the Western Atlantic Ocean, from the Gulf of Mexico and the Caribbean to Brazil has recently been divided in two different species, based on genetic and phenotypic traits: *A. bahianus* and *A. tractus* (Bernal and Rocha, 2011). Hence, the name *A. bahianus* is maintained for individuals from Brazil, as individuals from Western Atlantic Ocean, the Gulf of Mexico and the Caribbean Sea form a different species, *Acanthurus tractus* Poey, 1860, but retain the common name as ocean surgeonfish (Bernal and Rocha, 2011). Ocean surgeonfish (*A. tractus*) can be found 5 - 25 m deep and are identified by their yellow-greyish blue to dark brown body with no dark vertical bars, translucent pectoral fin with yellow tints, and a pale band on the base of the caudal fin. Additionally, they have a lunate caudal fin and measure 15 - 30 cm TL (Humann and Deloach, 2002). Ocean surgeonfish are considered relatively highly mobile (home range > 50 m^2), and usually are not-aggressive grazers (Ceccarelli *et al.*, 2005). Indeed, they spend most of their time foraging through the reef, consuming inorganic sediments with their algal food source (Wolf, 1987). The ocean surgeonfish is a gregarious species, forming loose single, small or large aggregated schools (Lawson *et al.*, 1999).

Both species are frequently infected by ectoparasites and are well known visitors to cleaning stations (Arnal and Côté, 1998; Wicksten, 1998; Arnal *et al.*, 2001; Cheney and Côté, 2001, 2003a; b; c; d, 2005; Côté and Molloy, 2003; Sikkel *et al.*, 2004, 2006, 2009; Soares *et al.*, 2007, 2008d; Coile and Sikkel, 2013).

1.6. STUDY OBJECTIVES

Based on the fact that ectoparasites are an inherent part of the ecosystem, two main objectives were proposed: (1) to understand how Curaçaoan reef conditions (such as loss of live coral and fish density) are affecting two reef fishes' ectoparasite loads; and (2) to understand how these parasite loads are influencing host behaviour, as clients toward cleaning.

2. ARE ECTOPARASITE LOADS OF REEF FISH INFLUENCED BY BENTHIC COVER AND FISH DENSITY?

2.1. INTRODUCTION

Coral reef degradation, with loss of live coral and increase in algal cover, is a problem worldwide, but the Caribbean is one of the most affected regions (Roff and Mumby, 2012). These changes in benthic community have repercussions in the global reef ecosystem, and several studies have mainly focused on the effect and relation with fish and corals (*e.g.* McClanahan *et al.*, 1999; Vermeij *et al.*, 2010a; Wild *et al.*, 2014; Hixon, 2015). Yet, the importance of an easily overlooked group in the ecosystem, the ectoparasites, cannot be ignored, as they are capable of influencing their host by affecting its behaviour, fitness and survival (Rosenqvist and Johansson, 1995; Finley and Forrester, 2003; Hudson *et al.*, 2006; Grutter *et al.*, 2011). Considering coral reefs, and especially with the increasing degradation (Wilkinson, 2000; Gardner *et al.*, 2003; Roff and Mumby, 2012), ectoparasites assume an even more relevant role, as several studies have suggested that sites associated with higher levels of reef degradation also have higher ectoparasite abundance (Grutter, 1998; Sikkel *et al.*, 2000, 2009; Sasal *et al.*, 2007).

In a recent study by Artim and Sikkel (2013), conducted in the Caribbean, it is concluded that not only the emergence rate of gnathiids (common ectoparasites with a benthic life stage) was lower in the presence of live coral, but also that gnathiids actively avoided contact with live coral, tolerating a wide range of alternative substrata (such as dead coral, sponge, algae, and even sand). Another study, aimed at the ectoparasite family branchiura, concluded that the percentage of infected fish was higher in vegetated microhabitats, as opposed to open habitats (Poulin and FitzGerald, 1989a). Moreover, there is evidence of the existence of more potential pathogens within areas with more turf algae (Casey *et al.*, 2014), which are among the most abundant benthic organisms in degraded reefs (Vermeij *et al.*, 2010b). Therefore, it seems accurate to assume there are favourable and unfavourable benthic compositions, at least as far as ectoparasite benthic stages are concerned.

Apart from the human impact on reef degradation, some reef fish also contribute to an increase in favourable substratum for ectoparasites. For instance, damselfish actively maintain algae mats in their territories, which serve as a permanent food source. However, these algae mats are maintained at the expense of coral growth and fitness (Vermeij *et al.*, 2010b; Hixon, 2015), whether by suffocating corals, blocking light (Nugues and Roberts, 2003), or by accumulating potential pathogens (Titlyanov *et al.*, 2007), increasing the occurrence of coral diseases (Casey *et*

al., 2014; Vermeij *et al.*, 2015). Hence, the decrease of live coral, facilitated by damselfish, gives way to algal colonization (Titlyanov *et al.*, 2007) and leads to an increase in favourable substratum for ectoparasites with benthic stages.

On the other hand, the hosts themselves influence the parasite community in a different way. Although it is important to realize that host-parasite interactions experience some level of specificity (Grutter, 1994; Lo *et al.*, 1998), and susceptibility (Coile and Sikkel, 2013), in a broader perspective, colonization opportunities for parasites depend, to some degree, on how many hosts are available in a certain area, that is, on host density (Morand and Poulin, 1998). This relation is especially true to directly transmitted parasites, *i.e.* ectoparasites (Arneberg *et al.*, 1998; Simková *et al.*, 2001). With an increase in density, there is an increase in fish proximity and, therefore, higher probability of contact with ectoparasites in their transmission stage (egg or larva) (Arneberg *et al.*, 1998; Arneberg, 2001, 2002; Finley and Forrester, 2003; Molinet *et al.*, 2011). So, it is expected that gregarious species (*e.g.* surgeonfish and parrotfish), have higher ectoparasite loads than solitary species (*e.g.* damselfish), which means that host's social behaviour may be an important factor to consider in ectoparasite studies (Caro *et al.*, 1997; Raibaut *et al.*, 1998; Sasal and Morand, 1998; Sasal, 2003; Bagge *et al.*, 2004). Additionally, highly mobile species also seem to harbour more ectoparasites (Caro *et al.*, 1997; Raibaut *et al.*, 1998; Sasal and Morand, 1998; Morand *et al.*, 2000). For instance, surgeonfish, that have a relatively large territory, showed to be disproportionally affected by dermal parasites in Curaçao, and the authors suggested their behaviour as gregarious foragers, frequently found near the bottom, as an explanation for these results (Bernal *et al.*, 2015).

Additionally to different infection opportunities, host susceptibility to ectoparasites vary with species, with individuals, and with changing environmental conditions (*e.g.* Lo *et al.*, 1998; Christe *et al.*, 2003; Sikkel *et al.*, 2009; Coile and Sikkel, 2013). Moreover, ectoparasites themselves play an active role as they show host preference (*e.g.* Yeo and Spieler, 1980; Grutter, 1994; Sikkel *et al.*, 2000; Nagel and Grutter, 2007). Considering that ectoparasites are affected by the micro- and macroenvironment (the host and its environment, respectively), it is important to study different species with distinct habits in order to better understand how reef degradation and loss of live coral may be affecting ectoparasite communities.

Although the Caribbean is one of the most affected regions by coral degradation, there are still sites, like Curaçao, where this degradation is occurring at a lower rate, and relatively healthy reefs can be found (Vermeij, 2012). The aim of the present study is to understand: (1) if fish from different reefs have different ectoparasite loads; and (2) if these ectoparasite loads are related

with reef degradation or fish density. Furthermore, differences between host species will also be explored in order to verify how social behaviour may influence ectoparasite loads.

2.2. MATERIALS AND METHODS

2.2.1. Study sites and Species

The study was carried out in Curaçao (12°N, 69°W), Netherlands' Antilles, Caribbean (Figure 1), between July and August 2014.
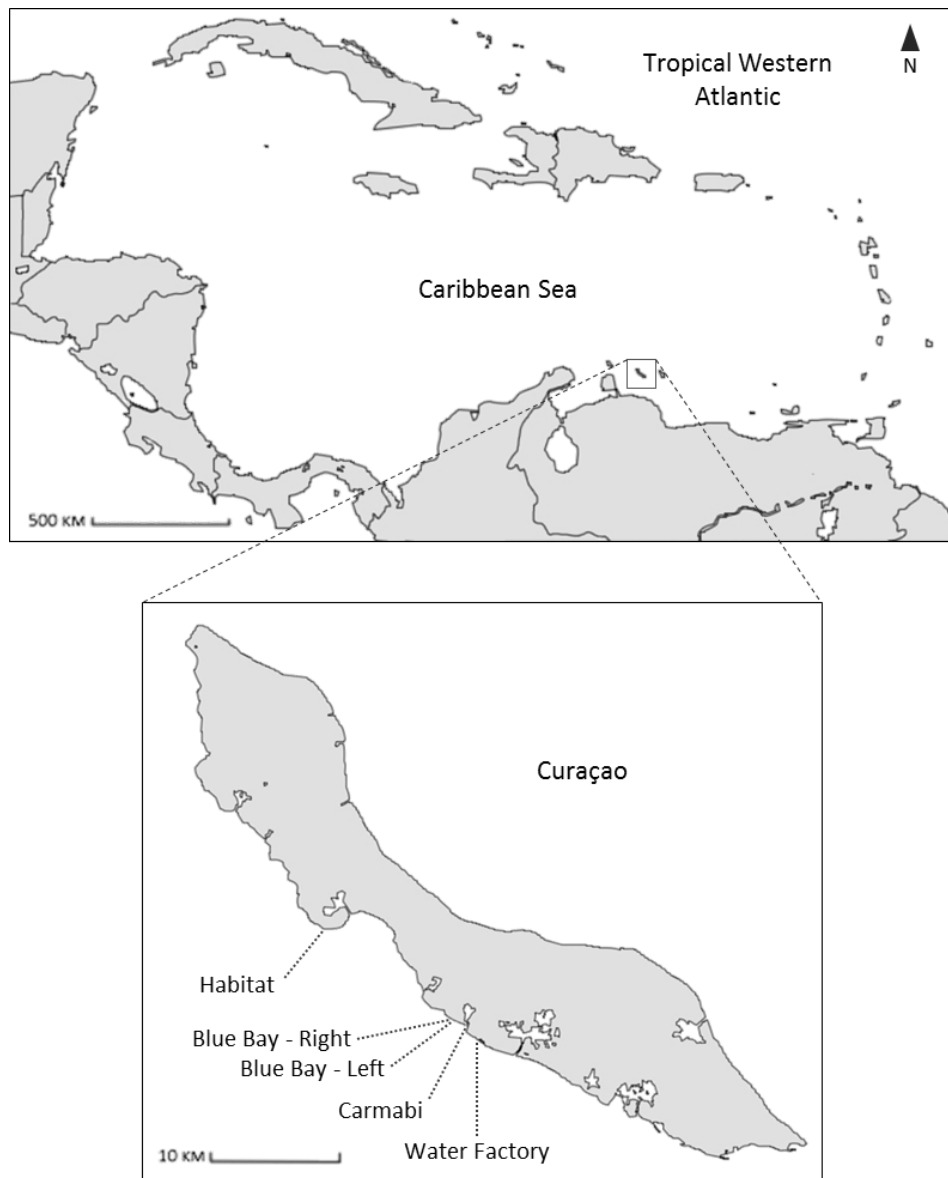
Figure 1 - Location of Curaçao in the Caribbean, and of the five reefs under study.

Sampling was done at five southwestern fringing reefs - Habitat, Blue Bay Right, Blue Bay Left, Carmabi and Water Factory (Figure 1). These reefs have approximately 60 m wide reef flat that gradually leads to a drop-off at 7-12 m deep, where the reef slope varies from 45° to 90° (Bak, 1975). These reef flats substrate typically consists of small coral colonies scattered throughout the sand, while coral density increases towards the drop-off (Bak, 1975). The most distant reef was Habitat (12 500 m to Blue Bay Right). Water Factory - Carmabi and Carmabi - Blue Bay Left were 2500 m and 2000 m apart, respectively. The closest reefs were Blue Bay Left and Right, separated by 300 m of sand. On each reef, the study area covered *ca*. 3000 m^2, at a maximum depth of 12 m, near the drop-off.

The present study focussed on two reef fish species: the longfin damselfish, *Stegastes diencaeus* (Jordan & Rutter, 1897), and the ocean surgeonfish, *Acanthurus tractus* Poey, 1860, chosen for their abundance, ease to identify and capture, and previous inclusion in several ectoparasite studies (*e.g.* Arnal *et al.*, 2001; Cheney and Côté, 2001, 2003d; Sikkel *et al.*, 2004, 2009; Soares *et al.*, 2007). Moreover, they strongly differ in home range size and social behaviour. While ocean surgeonfish are gregarious and have a relatively large territory (Lawson *et al.*, 1999), longfin damselfish are solitary, and actively defend their small well-defined territories (Cheney and Côté, 2003a).

2.2.2. Benthic and Fish community

The benthic community composition was assessed using line-intercept transects. Six 10 m long transects were followed haphazardly on each reef, parallel to the shore. Using a point-intercept method, the benthic cover type was recorded every 10 cm (total of 101 points per transect). Benthic cover included three categories: live coral, sand and other. Dead coral, algae and sponge were merged to create the category "other," as they may all be considered as marks of reef degradation and are favourable substrata for the benthic stages of ectoparasites (Coile and Sikkel, 2013). Each category was then expressed as the number of intercepted points falling into that category, divided by the total number of interception points per transect (Beldade *et al.*, 2015), and converted to percentage.

Fish density was assessed by undertaking ten transects per reef, parallel to the shore. Each transect was performed by two roving SCUBA divers that registered, on plastic slates, the number and species of fish within an area of approximately 4 m wide x 50 m long. Each diver was prepared to identify and register a variable set of fish families (determined *a priori*), to facilitate biodiversity assessment and to increase precision. Using the same transect, on the way back to

the starting point, each diver counted the number of *Elacatinus evelynae* (Böhlke & Robins, 1968) on his/her side of the transect line.

2.2.3. Ectoparasite load assessment

Ten to eleven individuals from each fish species under study were sampled in each reef. The procedure for fish capture and parasite load assessment was based on Grutter (1995), Sikkel *et al.* (2004), and Soares *et al.* (2007), and fish were collected whilst SCUBA diving between 10:00 and 17:00 hours. The same method was used to capture both fish species: a barrier net was first strategically placed on the reef flat. The fish were then individually herded towards the barrier net (barrier net size, for ocean surgeonfish: 6 m x 4 m long and 5.7 cm mesh size; and for longfin damselfish: 1.5 m x 2.5 m long and 2 cm mesh size). Each individual was then caught with a hand net as quickly as possible, and immediately placed into a hermetically sealed plastic bag filled with seawater. Each fish, including the contents of the respective bag, was then taken to the beach and placed into an individual container with a variable amount of seawater and 4 to 6 drops of clove oil (depending on fish size), a natural anaesthetic. After approximately 5 min, or when the opercular movements were visibly slower, each fish was then transferred to a bucket of fresh water, where it was measured and gently brushed to help remove the remaining ectoparasites. Finally, fish were placed in seawater-filled recovery containers and then released at the respective capture location. All fluids left in the containers were filtered using a plankton net (50 μm mesh size) and ectoparasites were preserved in 70% alcohol. In the laboratory, ectoparasites retrieved were counted and identified to family using a binocular microscope.

2.2.4. Statistical analysis

To examine differences in reef benthic community and in fish assemblage, an analysis of similarity (ANOSIM) was performed, using PRIMER 6, Primer-E, Ltd. Abundance matrices were compiled (using the categories for benthic community, and fish species for fish assemblage), and the data was square-rooted in order to compute a Bray-Curtis resemblance matrix. ANOSIM generates a R statistic between 0 - as much similarity within as between reefs - and 1 - all transects within reefs are more similar to each other than to any transect across reefs - which is tested for differences from zero with a permutation test (N_{max} = 999 permutations). The overall ANOSIM was followed by pairwise comparisons between reefs. To visualize these differences a multidimensional scaling (MDS) plot was used. Stress values lower than 0.1 suggest that distances among samples in an MDS plot accurately reflect the extent of community differences (Clarke and Warwick, 2001). To identify which reef pair had a higher percentage of dissimilarity in benthic community and fish assemblage a similarity percentage analysis (SIMPER) routine in PRIMER 6 was used.

Using IBM SPSS Statistics 22, differences among reefs were examined, always considering the two studied species separately. One-way ANOVA tests were performed for global fish density and followed by Tukey post-hoc tests, whenever differences were found. As parametric assumptions were not met for any other data set, Kruskal-Wallis tests were then performed, followed by Dunn´s post-hoc tests, whenever necessary. To control for potential size variations among hosts, in ectoparasite load analysis the number of ectoparasites was divided by fish total length (cm). Additionally, within each reef, differences between the ectoparasite loads of host species were investigated by the non-parametric Mann-Whitney test. Finally, to explore potential associations between the several ectoparasite loads, benthic community and fish density, Spearman correlations were used between each reef-specific mean values, whenever differences between reefs were found. Tests were always two tailed and performed after outlier exclusion.

2.3. RESULTS

2.3.1. Benthic community across reefs

Overall, significant differences between reefs were found when considering all benthic categories - live coral, sand and other (one-way ANOSIM, R = 0.40, P = 0.001). Except for Habitat - Blue Bay Left (P = 0.663) and Habitat - Blue Bay Right (P = 0.136), reef pairs significantly differed from each other (P < 0.005). Indeed, after a MDS was performed (Figure 2) Water Factory and Carmabi seemed to separate from the rest with less overlap.
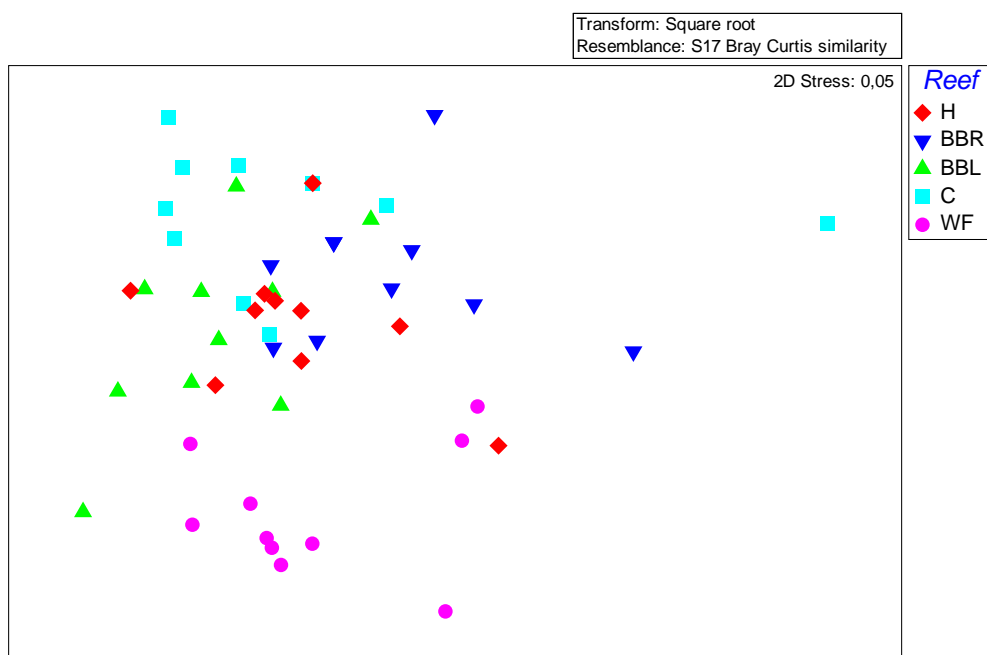
Figure 2 - Multidimensional scaling plot of benthic community across reefs. Each point corresponds to a single transect done in Habitat (H), Blue Bay Right (BBR), Blue Bay Left (BBL), Carmabi (C), and Water Factory (WF). Sample size: 10 transects per reef.

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As for Blue Bay Left and Blue Bay Right there was also a separation between the two, although not so obvious, since Habitat was overlapping with both reefs (Figure 2).

SIMPER also showed a higher benthic dissimilarity between Carmabi and Water Factory (46.94%), followed by Water Factory - Blue Bay Right (33.59%), Carmabi - Habitat (30.80%), Carmabi - Blue Bay Left (30.92%), Carmabi - Blue Bay Right (28.71%), Water Factory - Blue Bay Left (28.47%), Water Factory - Habitat (27.65%), and finally Blue Bay Left - Blue Bay Right (25.86%).

As for the evaluation of each category alone, there were also significant differences in the cover of live coral (Kruskal-Wallis, $H_4 = 29.70$, $P < 0.001$), the category other, including favourable substrate (Kruskal-Wallis, $H_4 = 24.42$, $P < 0.001$) and sand (Kruskal-Wallis, $H_4 = 14.10$, $P = 0.007$) (Figure 3). Post-hoc tests revealed that Water Factory had significantly higher live coral cover than all remaining reefs (Dunn's test, $P < 0.044$), and lower favourable substratum cover when compared to Blue Bay Left and Carmabi (Dunn's test, $P < 0.004$). Finally, Blue Bay Right had significantly higher amounts of sand cover than Blue Bay Left and Water Factory (Dunn's test, $P < 0.019$).
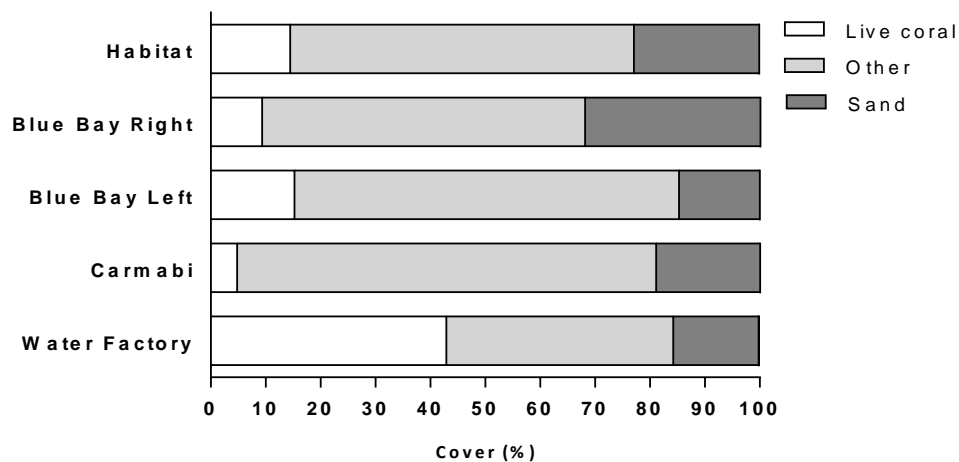
Figure 3 - Mean percentage cover of benthic categories across the five reefs. Sample size: 10 transects per reef.

2.3.2. Fish density and community across reefs

Overall species density was found to be significantly different amongst reefs (one-way ANOSIM, R = 0.51, $P = 0.001$) and between all reef pairs ($P < 0.002$). Transects from Blue Bay Left, Habitat and Water Factory seem to form relative cohesive groups (Figure 4). Although Blue Bay Right transects formed a relatively cohesive group, they partially overlapped Blue Bay Left and Habitat. Carmabi transects did not overlap those of other reefs, but showed a high heterogeneity among them (Figure 4).
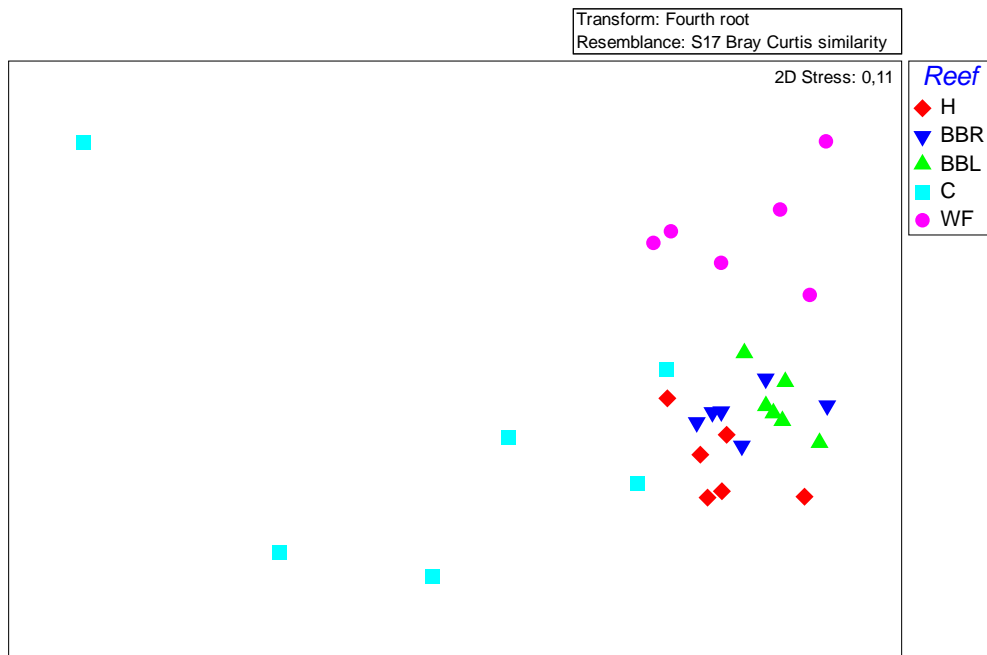
Figure 4 - Multidimensional scaling plot considering each species density across reefs. Each point corresponds to a single transect done in Habitat (H), Blue Bay Right (BBR), Blue Bay Left (BBL), Carmabi (C), and Water Factory (WF). Sample size: 10 transects per reef.

Through SIMPER analysis, the highest dissimilarity was found between Carmabi and Water Factory (45.82%), followed by Carmabi - Blue Bay Left (41.52%), Carmabi - Habitat (41.02%), Carmabi - Blue Bay Right (40.61%), Water Factory - Habitat (35.64%), Water Factory - Blue Bay Right (33.51%), Water Factory - Blue Bay Left (33.22%), Habitat - Blue Bay Left (27.99%), Habitat - Blue Bay Right (24.70%), and finally Blue Bay Right - Blue Bay Left (24.02%).

Global fish density, considering individuals from all 88 species registered, was found to be significantly different amongst reefs (one-way ANOVA, $F_{4, 22} = 10.33$, $P < 0.001$). Specifically, they differed significantly between Carmabi-Blue Bay Left, -Blue Bay Right, and -Habitat, and between Water Factory-Blue Bay Left and -Blue Bay Right (Tukey test, $P < 0.025$). For all the paired comparisons mentioned, Carmabi (mean ± SD = 4.31 ± 2.33 ind. m^{-2}) and Water Factory (mean ± SD = 6.04 ± 1.15 ind. m^{-2}) had the lowest global fish density (Figure 5).
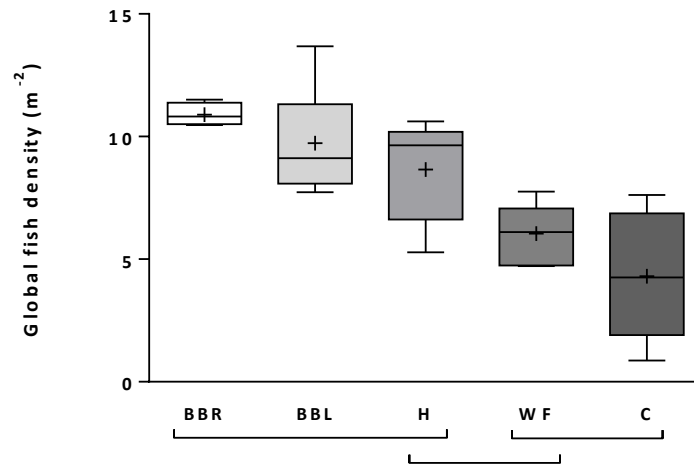
Figure 5 - Global fish density (ind. m^{-2}) in Blue Bay Right (BBR), Blue Bay Left (BBL), Habitat (H), Carmabi (C), and Water Factory (WF). Box-plots represent median, minimum and maximum and 1st and 3rd quartiles. Mean marked as "+". Sample size: $n_{BBR} = 4$, $n_H = 5$, $n_{BBL} = n_{WF} = n_C = 6$. Lines below the graph group reefs with similar global fish density.

Finally, regarding the two studied fish species' density, differences were only found to be significant for longfin damselfish (Kruskal-Wallis, $H_4 = 14.23$, $P = 0.007$), with lower density in Carmabi when compared with Blue Bay Left (Dunn's test, $P = 0.006$) (Figure 6). On the other hand, ocean surgeonfish density was similar across reefs (Kruskal-Wallis, $H_4 = 8.89$, $P = 0.064$) (Figure 6).
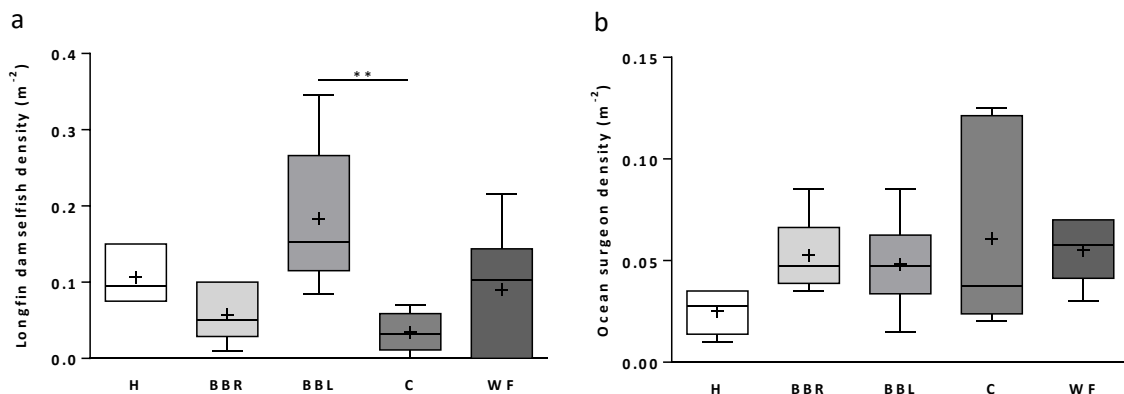
Figure 6 - Density (ind. m^{-2}) of a) longfin damselfish and b) ocean surgeonfish in Habitat (H), Blue Bay Right (BBR), Blue Bay Left (BBL), Carmabi (C), and Water Factory (WF). Box-plots represent median, minimum and maximum and 1st and 3rd quartiles. Mean marked as "+". Sample size of 6 transects in each reef. Significant differences between reef pair marked: ** Dunn's test, $P < 0.010$.

2.3.3. Ectoparasite loads across reefs

From a total of 102 fish collected, 22 had no ectoparasites (20 damselfish and only 2 surgeonfish). Total ectoparasite loads significantly differed at least between two reefs for longfin damselfish (Kruskal-Wallis, H_4 = 10.128, *P* = 0.038), but paired post-hoc analysis did not find reefs differing significantly (Dunn's test, P > 0.060 for all reef pairs) (Figure 7a). For ocean surgeonfish ectoparasite loads were similar across reefs (Kruskal-Wallis, H_4 = 3.30, *P* = 0.509) (Figure 7b).
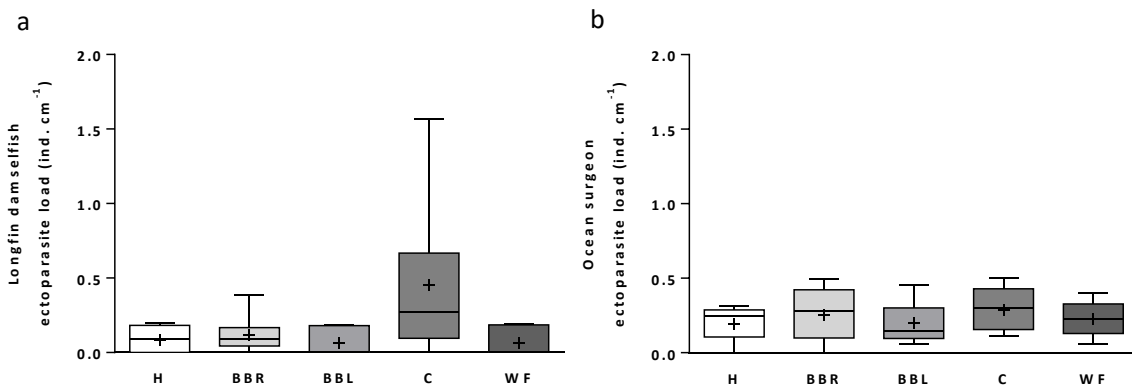
Figure 7 - Ectoparasite load (per cm of TL) of a) longfin damselfish and b) ocean surgeonfish in Habitat (H), Blue Bay Right (BBR), Blue Bay Left (BBL), Carmabi (C), and Water Factory (WF). Box-plots represent median, minimum and maximum and 1st and 3rd quartiles. Mean marked as "+". Sample size respectively for longfin damselfish and ocean surgeonfish: n_{BBR} = 9, n_{BBL} = n_H = n_{WF} = 10, n_C = 11 and n_{BBR} = n_{BBL} = n_C = n_{WF} = 10, n_H = 11.

Ectoparasites retrieved included gnathiid isopods (Gnathiidae), caligid copepods (Caligidae) and capsalid monogeneans (Capsalidae) (Appendix I). On 51 longfin damselfish a total of 33 gnathiids, 26 caligids (none in Habitat) and 38 capsalids (34 just in Carmabi) were collected. As for the 51 ocean surgeonfish a total of 165 caligids were retrieved, along with 24 gnathiids and 13 capsalids (11 in Carmabi).

As it can be seen in Table 1, all ectoparasite family loads showed some significant differences between reefs in longfin damselfish (per centimetre of host) (Kruskal-Wallis: gnathiids, H_4 = 9.73, *P* = 0.045; caligids, H_4 = 10.09, *P* = 0.039; capsalids, H_4 = 21.90, *P* < 0.001). However, post-hoc tests were only capable of revealing differences in capsalid loads, with longfin damselfish collected in Carmabi scoring the highest loads (median ± interquartile = 0.10 ± 0.45 ind. cm^{-1}) when compared with all other reefs (Dunn's test, *P* = 0.003). As for ocean surgeonfish, caligid loads were similar between reefs (Kruskal-Wallis, H_4 = 1.42, *P* = 0.842), unlike gnathiid and capsalid loads (Kruskal-Wallis: gnathiids, H_4 = 11.81, *P* = 0.019; capsalids, H_4 = 13.56, *P* = 0.009). Once again, post-hoc test solely revealed significant differences for capsalid loads, with Carmabi having ocean surgeonfish

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with the highest infestation levels (median ± interquartile = 0 ± 0.06 ind. cm^{-1}) compared with the remaining reefs (Dunn's test, $P < 0.039$).

Table 1 - Mean and maximum ectoparasite family loads per host species in each reef. Zeros represent samples in which the only infected fish was considered an outlier, and were thereby not considered, and "-" represent samples where all fish collected had no ectoparasites. Significant differences marked: ns $P > 0.050$, * $P < 0.050$, ** $P < 0.010$, *** $P < 0.001$

	Habitat Mean (max.)	Blue Bay Right Mean (max.)	Blue Bay Left Mean (max.)	Carmabi Mean (max.)	Water Factory Mean (max.)	Significance between reefs
Gnathiid loads						
Longfin damselfish	0.076 (0.19)	0.073 (0.19)	0.022 (0.11)	0.080 (0.34)	0	*
Ocean surgeonfish	0.022 (0.06)	0	0	0.040 (0.10)	0.029 (0.06)	*
Significance between species	ns	*	ns	ns	ns	
Caligid loads						
Longfin damselfish	-	0.073 (0.37)	0	0.103 (0.52)	0	*
Ocean surgeonfish	0.172 (0.29)	0.224 (0.40)	0.175 (0.45)	0.182 (0.44)	0.191 (0.36)	ns
Significance between species	***	*	**	ns	**	
Capsalid loads						
Longfin damselfish	0	0	0	0.269 (1.13)	0	***
Ocean surgeonfish	-	0	-	0.034 (0.19)	0	**
Significance between species	ns	ns	ns	ns	ns	

Within each reef, total ectoparasite loads between the two host species were similar in Blue Bay Right (Mann-Whitney, $U_{17} = 24.00$, $P = 0.095$) and Carmabi (Mann-Whitney, $U_{19} = 60.00$, $P = 0.756$), but they revealed to be different in Habitat (Mann-Whitney, $U_{19} = 20.00$, $P = 0.013$), Blue Bay Left (Mann-Whitney, $U_{18} = 20.50$, $P = 0.023$) and Water Factory (Mann-Whitney, $U_{18} = 13.00$, $P = 0.004$) (Table 1). As for ectoparasite family loads the results were relatively consistent, with few exceptions, between host species within reefs (Table 1). Longfin damselfish gnathiid loads were higher than ocean surgeonfish loads in Blue Bay Right (Mann-Whitney,

$U_{16} = 68.00$, $P = 0.012$), but similar in the other reefs (Mann-Whitney: Habitat, $U_{19} = 80.00$, $P = 0.085$; Blue Bay Left, $U_{15} = 44.00$, $P = 0.481$; Carmabi, $U_{19} = 59.00$, $P = 0.809$; Water Factory, $U_{16} = 20.00$, $P = 0.083$). Except in Carmabi (Mann-Whitney, $U_{19} = 33.50$, $P = 0.132$), ocean surgeonfish caligid loads were always higher than longfin damselfish loads (Mann-Whitney: Habitat, $U_{19} = 5.00$, $P < 0.001$; Blue Bay Right, $U_{18} = 20.50$, $P = 0.023$; Blue Bay Left, $U_{16} = 8.00$, $P = 0.003$; Water Factory, $U_{16} = 4.00$, $P = 0.001$). Finally, host species capsalid loads were similar in all reefs (Mann-Whitney: Habitat, $U_{18} = 49.50$, $P > 0.050$; Blue Bay Right, $U_{16} = 40.50$, $P > 0.050$; Blue Bay Left, $U_{17} = 45.00$, $P > 0.050$; Carmabi, $U_{18} = 68.00$, $P = 0.175$; Water Factory, $U_{16} = 40.50$, $P > 0.050$).

2.3.4. Benthic community and fish density influence on fish ectoparasite loads

A negative correlation was found between longfin damselfish's total ectoparasite loads and live coral cover (Spearman correlation, $r_s = -0.90$, $n = 5$, $P = 0.037$) (Figure 8a).
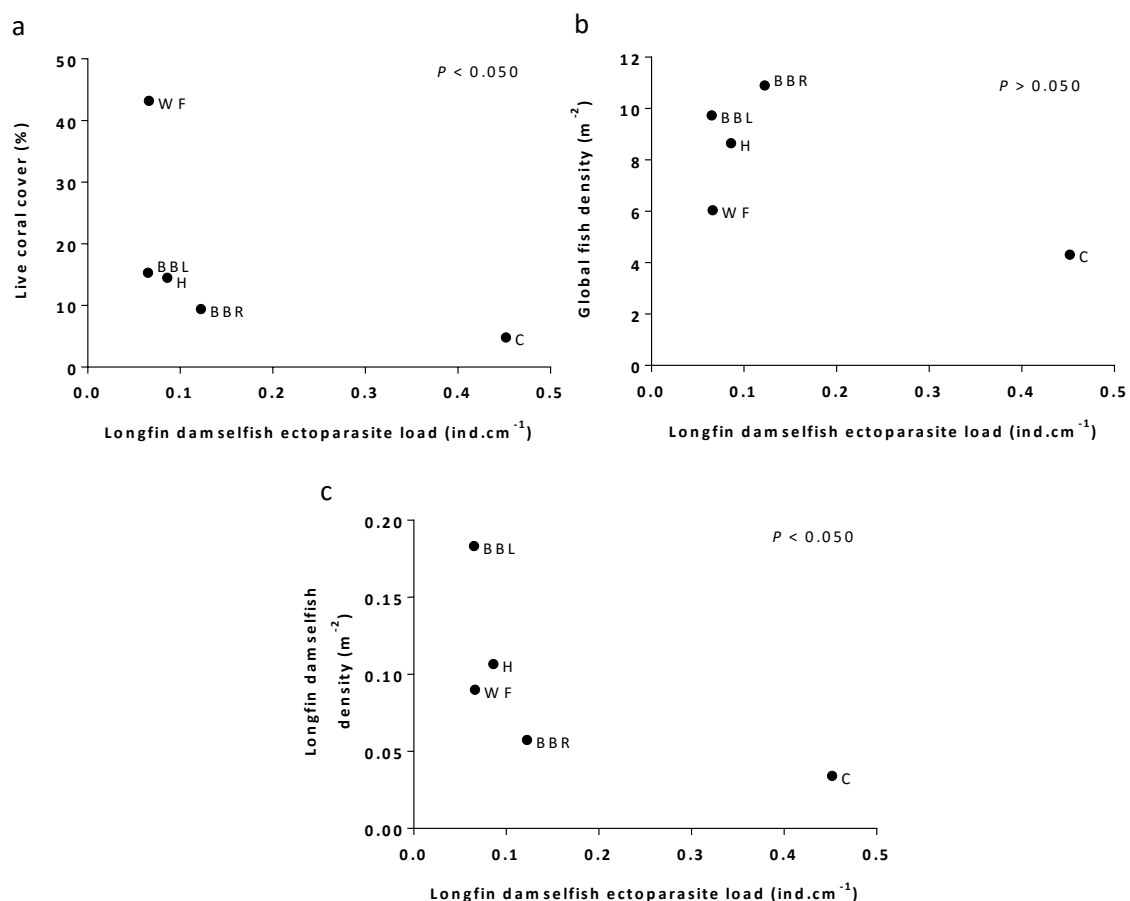
Figure 8 - Longfin damselfish ectoparasite loads per host centimetre in relation with a) live coral cover (%), b) global fish density (per m²), and c) their longfin damselfish density (per m²). Sample size: for all n = 5. Habitat (H), Blue Bay Right (BBR), Blue Bay Left (BBL), Carmabi (C), and Water Factory (WF).

Global fish density did not seem to be correlated with longfin damselfish ectoparasite loads (Spearman correlation, $r_s = -0.30$, n = 5, P = 0.624) (Figure 8b). Finally, longfin damselfish ectoparasite loads were found to be negatively associated with density (Spearman correlation, $r_s = -0.90$, n = 5, P = 0.037) (Figure 8c).

The same correlations that were performed for total ectoparasite load were also done separately per ectoparasite family. Regarding longfin damselfish, a negative correlation with live coral cover was found for gnathiid loads (Spearman correlation, $r_s = -0.90$, n = 5, P = 0.037), but not for capsalid loads (Spearman correlation, $r_s = -0.71$, n = 5, P = 0.182). None of the specific ectoparasite family loads of longfin damselfish were associated with global fish density (Spearman correlation (n = 5): gnathiids, $r_s = -0.30$, P = 0.62; caligids, $r_s = -0.22$, P = 0.718; capsalids, $r_s = -0.71$, P = 0.182). Only caligids were negatively correlated with longfin damselfish density (Spearman correlation (n = 5): gnathiids, $r_s = -0.50$, P = 0.391; caligids, $r_s = -0.89$, P = 0.041; capsalids, $r_s = -0.71$, P = 0.182) (Table 2).

Table 2 - Spearman correlations of longfin damselfish ectoparasite loads (ind. per TL cm), regarding ectoparasite family with live coral cover (%), global fish density (per m^2), and single species density (per m^2). Sample size: all correlation n = 5. Significant correlations in boldface and marked: * P < 0.050.

Correlation		r_s	P
Live coral cover	Gnathiid loads	**−0.90**	**0.037***
	Capsalid loads	−0.71	0.182
Global fish density	Gnathiid loads	−0.30	0.624
	Capsalid loads	−0.71	0.182
	Caligid loads	−0.22	0.718
Longfin damselfish density	Gnathiid loads	−0.50	0.391
	Capsalid loads	−0.71	0.182
	Caligid loads	**−0.89**	**0.041***

As for ocean surgeonfish, no correlation was found in relation to each ectoparasite family loads with live coral cover (Spearman correlation (n = 5): gnathiids, $r_s = -0.21$, P = 0.741; caligids, $r_s = -0.10$, P = 0.873; capsalids, $r_s = -0.71$, P = 0.182). Interestingly, gnathiid loads were found to be negatively correlated with global fish density (Spearman correlation (n=5): gnathiids, $r_s = -0.98$, n = 5, P = 0.005; capsalids, $r_s = -0.71$, P = 0.182) (Table 3).

Table 3 - Spearman correlations of ocean surgeonfish ectoparasite loads (ind. per cm), regarding ectoparasite family with live coral cover (%), global fish density (per m²). Sample size: all correlation n = 5. Significant correlations in boldface and marked: ** $P < 0.010$.

Correlation		r_s	P
Live coral cover	Gnathiid loads	−0.21	0.741
	Capsalid loads	−0.71	0.182
Global fish density	Gnathiid loads	**−0.98**	**0.005****
	Capsalid loads	−0.71	0.182
	Capsalid loads	−0.71	0.182

2.4. DISCUSSION

Curaçao is known to have healthier reefs relatively to other islands in the Caribbean (Vermeij, 2012; Jackson *et al.*, 2014), however these are still found to be degraded (Bak *et al.*, 2005; Vermeij, 2012). Indeed, Curaçao proved to be a good location for studies in which different reef degradation levels are required. Although ectoparasite loads did not differ as much as expected between reefs, it still could be found that fish species were differently infected by ectoparasites. Furthermore, it is important to consider ectoparasite families separately as they were affected by different reef characteristics.

2.4.1. Reef characterization

Two of the sampled reefs (Carmabi and Water Factory) demonstrated to be the most distinct from each other, when considering both benthic categories and overall species density. In a report in which these sites were included (Vermeij, 2012), Carmabi was described to be experiencing severe degradation and no longer forming or renewing the existing reef structure. Conversely, Water Factory was one of the few sites where reef structures were still forming, but it was not categorized as a healthy reef due to be under moderate degradation rates (rather than fast or severe). Additionally, the report also mentioned Habitat reef as subjected to a fast degradation rate and no longer forming reef structures, but with a still slightly higher calcification index than Carmabi (Vermeij, 2012).

In terms of global fish density (including individuals from all 88 species registered) however, both Carmabi and Water Factory shared the lowest values. As fish density is usually predicted to increase with higher live coral cover (Bell and Galzin, 1984), this result was not expected for

Water Factory, since it presented the highest live coral cover values. This may partially be explained by reef location. During diving sessions several recreational fishermen were seen fishing in Carmabi, and in Water Factory the reef was close to a fishing dock, which can contribute to the scarcity of fish in this spot. Additionally, a local diving instructor reported that in Water Factory existed a second reef flat further from shore, with better reef quality and thus higher fish density. This alternative habitat with better conditions may be driving most of the fish community there. Regarding the studied species density, the only variation found was in longfin damselfish density between Blue Bay Left and Carmabi, with the latter revealing lower values.

2.4.2. Ectoparasite loads

When both host species were considered, overall ectoparasite loads did not differ much between reefs. The only significant difference was found in Carmabi, where longfin damselfish ectoparasite loads seemed to be higher than in the other reefs. This may indicate that differences between the sampled reefs were not sufficient to distinctly affect ectoparasite incidence. However, the question remained as to whether these differences in ectoparasite loads were linked to specific reef characteristics, such as live coral cover. Indeed, ectoparasite loads on longfin damselfish were higher in reefs with lower live coral cover and with lower longfin damselfish density. However, pooling together all ectoparasite families excludes from consideration their biologic and ecologic differences, which may be key factors influencing general trends. In the present study, three common ectoparasite families were represented: Gnathiidae, Caligidae and Capsalidae. Capsalids are not unusually found on fish, but gnathiids and caligids are the two most commonly found ectoparasite families (Bunkley-Williams and Williams, 1998; Grutter, 2002). Gnathiid isopods, the most well studied ectoparasites, have a parasitic phase only as larvae, during which they emerge from the substrate to find a suitable host, upon which they feed from 2-4 h to several days (Grutter, 1998) before returning to the benthos to moult into the next phase. The same process is repeated three times until they moult into non-parasitic adults (Smit and Davies, 2004). Capsalid monogeneans are oviparous with benthic eggs. Eggs hatch into active swimming larvae, a process that may be enhanced by host signals and cues. Finally, to our knowledge, caligid copepods have no dependence on the benthos. Instead, host seeking caligids are normally found closer to the water surface rather than the bottom of the reef (Mordue (Luntz) and Birkett, 2009).

2.4.2.1. Gnathiidae

With one exception, host species had similar gnathiid loads in almost all reefs. So, it was not surprising to see that both species had Carmabi, presumably, as the reef where fish were the

most infected. However, gnathiid loads correlated with different reef characteristics depending on host species. Mean gnathiid loads on longfin damselfish were higher in reefs with lower mean live coral cover. For gnathiids, there is direct evidence of their repulsion for live coral (Artim and Sikkel, 2013). Considering that longfin damselfish have a close relation to their territories, and therefore to the benthos, in which they reduce live coral cover to maintain algal mats (Hixon, 2015; Vermeij *et al.*, 2015), they may become more susceptible to gnathiid infestation, since their territories have more favourable substrate for gnathiids. However, a more appropriate way to find a direct relation between these two variables would be to sample the benthic cover within fish territories.

Regarding ocean surgeonfish' gnathiid loads, a negative relation with live coral cover would also be expected, since they spend ca. 85% of their time foraging (Wolf, 1987), which implies a proximity to the substratum. In turn, it was found that mean ocean surgeonfish gnathiid loads were higher in reefs where, in average, there were less ocean surgeonfish per square meter. This relation was surprising considering that previous studies have found links between higher fish densities and higher ectoparasite loads (Morand and Poulin, 1998; Arneberg, 2001; Sasal, 2003). However, because gnathiids are mobile parasites and act as predators, potential hosts seem to make use of their group numbers to create a dilution effect, which may protect them against mobile parasites (Poulin and FitzGerald, 1989b; Côté and Poulin, 1995), but not necessarily against other types of parasites. Additionally, because ocean surgeonfish are mobile fish, they may "migrate" in order to avoid more infected areas, as it happens with other species (Welicky and Sikkel, 2015).

Thus, it was interesting to verify that gnathiid loads related to different variables according to host species, which may reflect an influence of host social behaviour, but also host specificity or vulnerability (Buchmann and Lindenstrøm, 2002; Sasal *et al.*, 2005; Grutter *et al.*, 2011; Sikkel *et al.*, 2011; Coile and Sikkel, 2013).

2.4.2.2. Capsalidae

Capsalid monogenean loads were relatively similar among host species in every reef, except for Carmabi, which had higher infection levels. Moreover, in other reefs the presence of capsalid monogeneans was rare, which may explain the lack of a correlation with either live coral cover or fish density. Yet, the absence of correlation was unexpected, as other studies found higher monogenean abundance in sites associated with lower live coral cover (Grutter, 1998; Sikkel *et al.*, 2000, 2009), and some of them even included the same host species as the present work. In

those studies, the differences were related or compared with distance from shore: higher monogenean loads were present when closer to shore. Actually, sites closer to shore tend to have worse water quality, either by an input of nutrients, sediments or even pollutants (Nugues and Roberts, 2003; Sasal *et al.*, 2007). Water quality is, indeed, a main abiotic variable that has been linked with monogenean infection in captive and wild hosts (Sikkel *et al.*, 2009). Moreover, when comparing capsalid monogeneans among sites, Sikkel and colleagues (Sikkel *et al.*, 2009) found highly infected surgeonfish within bays (rather than non-bay sites), where reef habitats were more degraded and had less water circulation. In light of this new perspective, Carmabi seems to have perfect conditions to be a monogenean hotspot, when compared with other reefs, due to the presence of a canal near the dive site. Indeed, in several dives this canal's water influx effect could be seen with the naked eye, as a fog-like cloud underwater. However, it is important to emphasize that no real data was collected, and for that this stands as a hypothesis that remains to be confirmed. Thereby, it is possible that monogeneans could be affected to a certain degree by water quality rather than substratum cover or hosts density. Additionally, an input of nutrients enhances algal growth (McCook, 1999), giving them an edge in space competition with live coral (Titlyanov *et al.*, 2007) Also, the influx of sediments in reefs leads to coral death, either by suffocation or by the impact in coral-algae interaction (facilitates algae overgrowth) (Nugues and Roberts, 2003). Thus, water quality might be enhancing monogenean and algae abundance, which in turn may retain monogenean in the reef.

2.4.2.3. Caligidae

Overall, ocean surgeonfish had more caligids per unit of fish length than longfin damselfish. Differences between species were consistent with data from other studies, in which damselfish usually do not harbour a high number of caligids (Arnal *et al.*, 2001; Cheney and Côté, 2001). Raibaut *et al.* (1998), found that gregarious fish (*e.g.* surgeonfish) have a higher parasitation index than solitary fish (*e.g.* damselfish), since during their infective stage parasitic copepods are able to swim actively, and host gathering further increases the encounter probability between ectoparasite and host (see also Arneberg *et al.*, 1998).

Ocean surgeonfish's caligid loads were similar across reefs, while longfin damselfish caligid loads pointed to the existence of some differences: mean infection loads were higher in reefs with lower longfin damselfish density, on average. This relation relative to longfin damselfish was less clear since they are solitary fish. However, sites with a higher longfin damselfish abundance could be providing better nourishment and as such, the hosts would potentially resist more efficiently to caligid infestation (Landolt, 1989). On the other hand, longfin damselfish can be healthier due

to lower caligid loads. Yet, perhaps a more parsimonious possibility, may be simply related to the fact that caligids are mobile parasites, and as gnathiids, a dilution effect ensures that the probability of a host being picked by the ectoparasite decreases with host density (Côté and Poulin, 1995).

2.4.3. Final remarks

The present study points for the fact that fish from different reefs varied in ectoparasite loads, and those loads were related with different reef variables according to host species and ectoparasite family. Solitary longfin damselfish infection seems to be more severe in reefs with less live coral cover (gnathiids) and worse water quality (capsalids). As for gregarious ocean surgeonfish infection by ectoparasites, this seems to be affected by fish density (caligids) and also by water quality (capsalids).

Although the recognition of ectoparasite as an important part of the ecosystem has been increasing over the past years, more conclusive studies are needed in order to understand what causes ectoparasite variation between different locations, but also throughout different time scales (daily and yearly), and even between different hosts.

3. ARE LEVELS OF PARASITISM RESPONSIBLE FOR DIFFERENCES IN CLEANING BEHAVIOUR OF REEF FISHES?

3.1. INTRODUCTION

The aggravation of reef degradation is changing the ecosystem, rapidly approaching a point of no recovery (*e.g.* Wilkinson, 2000; Gardner *et al.*, 2003; Pandolfi *et al.*, 2003; Vermeij, 2012). In this context, ectoparasites should be considered as double players, as they are influenced by the environment (*e.g.* Grutter, 1998; Barber *et al.*, 2000; Sikkel *et al.*, 2000; Grutter *et al.*, 2011; Artim and Sikkel, 2013) and in turn affect their hosts (Barber *et al.*, 2000). Indeed, a vast array of fish behaviours, such as reproduction, habitat selection, locomotion, foraging, avoidance of predation and of infected prey, and finally infection reduction behaviour, are influenced by ectoparasites, (Barber *et al.*, 2000; Hudson *et al.*, 2006). As for infection reduction, fish may actively search for ways to facilitate the removal of irritation sources (*e.g.* loosened scales, damaged tissue), or to reduce the load or severity of infection (*e.g.* ectoparasites) (Wyman and Walters-Wyman, 1985). While chafing may be a simpler way to deal with parasites, when an individual scrapes its body against an available substrate in an attempt to clean itself (Wyman and Walters-Wyman, 1985), interacting with cleaners should become a more efficient and beneficial way to reduce ectoparasite loads and the respective stress responses (Becker and Grutter, 2004; Ros *et al.*, 2011; Soares *et al.*, 2011).

Cleaning interactions are conspicuous activities that involve two parties: cleaners and clients. In coral reefs, cleaners are small fish or shrimp that remove ectoparasites, damaged tissue, scales and mucus from the body surface, buccal cavity and gills of usually larger fish, known as clients (Losey, 1974; Côté, 2000; Becker and Grutter, 2004). Client-cleaner interactions usually take place at specific sites called cleaning stations (Côté, 2000), where visiting clients seeking cleaning service often adopt a stereotyped and immobile posture in front of the cleaner, while waiting to be inspected (Losey, 1972). This posing behaviour may enhance the client's likelihood to be inspected by the cleaner, however clients may also leave the site without being inspected. However, even if the client turns out to be inspected, this inspection may vary in terms of quality (Côté *et al.*, 1998). For instance, a poorer service may turn out to be slowly initiated, incomplete and/or dishonest, *i.e.* an interaction in which the cleaner does not inspect the client properly, and/or in which the cleaner eats healthy tissue and mucus of the client (Bshary and Noë, 2003). Usually, the dishonest behaviour (also referred to as cheating) is visually translated by client jolts as a reaction to the removal of the items mentioned above by the cleaner (Soares *et al.*, 2008b).

The direct impact of ectoparasite depletion on clients has been linked to relief (Kanenko II *et al.*, 1988); decrease in transmission rate of other pathogenic organisms, promoted by ectoparasites (Davies and Smit, 2001; Panek, 2005; Cook *et al.*, 2015); improved body condition, since it tends to be reduced in the presence of ectoparasites (Ros *et al.*, 2011); and decrease in stress levels (Bshary *et al.*, 2007; Soares *et al.*, 2011; Sun *et al.*, 2015). However, it has been difficult to understand how changes in parasite availability may or may not influence clients' specific motivation to interact with cleaners. Some studies point towards ectoparasite loads being not significant in cleaners' seeking behaviour (Losey, 1972; Grutter, 1996; Côté and Molloy, 2003) while others show ectoparasites as an important variable to explain cleaning interactions (Grutter, 1995; Sikkel *et al.*, 2000; Côté and Molloy, 2003; Cheney and Côté, 2005), both as proximate, and sometimes as the ultimate cause (Arnal and Morand, 2001; Arnal *et al.*, 2001; Grutter, 2001; Cheney and Côté, 2003b; Sikkel *et al.*, 2004). In such studies, clients have been found to invest more in visiting cleaning stations when and where ectoparasite loads are higher, posing for and being inspected by cleaners more often and for longer periods of time (see Grutter, 1995; Sikkel *et al.*, 2000; Côté and Molloy, 2003; Cheney and Côté, 2005). Hence, it would be expected that more parasitized clients would have a higher tolerance for lower quality cleaning service, as the benefits of ectoparasite removal outweighed the interaction's costs.

However, the rather high controversy concerning the role of ectoparasites as drivers of fish behaviour may be due to other important factors acting simultaneously. Indeed, fish with fresh wounds, and not necessarily heavily parasitized, spend more time at cleaning stations than those in a more advanced stage of the healing process (Foster, 1985).

Another irritation source may be related to other types of parasitism. Recently, two studies found a disproportional incidence of black blemishes on fish, especially the ocean surgeonfish (*Acanthurus tractus*), from Curaçao and Bonaire, with ca. 20% and 57% of infected individuals, respectively (Bernal *et al.*, 2015; de Graaf and Simal, 2015). Such blemishes are usually associated with dermal parasites (*e.g.* digenean metacercaria, turbellarians, and/or protozoan infections) (Potts, 1973; Bernal *et al.*, 2015), and cleaners have been observed attempting to feed on these blemishes on the client (Rosenqvist and Johansson, 1995). Loss of live coral, algae proliferation and decrease in water quality (all associated with reef degradation) may serve as an additional irritation vector to fish, as it may also facilitate conditions to the proliferation of some ectoparasites and other pathogens (Grutter, 1998; Sikkel *et al.*, 2000, 2009).

The role and impact of parasites may also be variable according to species. Not all species are the same when it comes to parasite incidence and disease vulnerability, nevertheless not much

information is available on this subject (Coile and Sikkel, 2013). Both Pomacentridae and Acanthuridae have an important role in coral reef ecosystem as herbivores (McClanahan *et al.*, 1999; Ceccarelli *et al.*, 2005; Casey *et al.*, 2014) and are relatively well known client fishes (Arnal *et al.*, 2000; Soares *et al.*, 2007, 2008a), which, in some places, have been found to increase their efforts towards cleaning according to ectoparasite load (Arnal *et al.*, 2001; Cheney and Côté, 2001; Sikkel *et al.*, 2004).

In this context, the aim of the present study was to test if differences in reef degradation and consequent variation on longfin damselfish (*Stegastes diencaeus*) and ocean surgeonfish (*Acanthurus tractus*) ectoparasite loads have an influence on client motivation and behaviour to engage in cleaning activities. Specifically, it was investigated (1) whether heavily infected individuals inhabiting these distinct reefs would seek cleaning differently (by chafing or by interacting with cleaners), (2) whether service quality provided to clients would vary, and (3) whether parasitation differently influences client species regarding cleaning behaviour.

3.2. MATERIALS AND METHODS

3.2.1. Study site and Species

The study was conducted in Curaçao (12°N, 69°W), Netherlands Antilles, Caribbean, in July and August 2014. In a previous study (see Chapter 2) the reefs at Carmabi and Water Factory, were considered as the two most different reefs in terms of their fish assemblage (45.82% dissimilarity) and benthic community (46.94% dissimilarity). These reefs were also the two extremes in terms of live coral cover. Thus, Carmabi, with ca. 5% live coral cover, was considered as the degraded reef, while Water Factory, with ca. 43% live coral cover, the less degraded reef (Appendix II). For these reasons Carmabi and Water Factory reefs were selected to conduct the present study.

3.2.2. Cleaner fish density

Cleaner fish density in the two selected reefs (Carmabi and Water Factory) was determined in a previous study (see Chapter 2). Four cleaner fish species were reported: sharknose goby, *Elacatinus evelynae*; yellownose goby, *Elacatinus randalli*; bluehead wrasse, *Thalassoma bifasciatum*; and Spanish hogfish, *Bodianus rufus*. However, yellownose gobies and Spanish hogfish were rare, *i.e.* not statically different from zero (one-sample Wilcoxon: yellownose goby, Water Factory, $W_6 = 3.00$, $P = 0.50$; Spanish hogfish, Carmabi, $W_6 = 1.00$, $P > 0.05$ and Water Factory, $W_6 = 3.00$, $P = 0.500$) or absent (namely yellownose goby in Carmabi). For this reason, these species were not considered in the present study. Additionally, bluehead wrasse acts as a cleaner only as a juvenile, but as their initial and terminal phases were not recorded

separately during sampling transects, the species density determined was considered as approximate to cleaner phase density.

3.2.3. Behavioural observations

Observations were made between 10:00 and 17:00 hours, through 20 min focal-follow video recordings of 9 - 11 individual clients, per focus species, in each reef. Focal clients were selected haphazardly and observations began immediately upon sighting, at a minimum distance of 2 m (Soares *et al.*, 2007). All ocean surgeonfish were observed alone rather than in school context, in order to facilitate observation. During video analysis, all visits to cleaning stations were noted, regardless of cleaner species. Specifically, for each interaction the following information was recorded: (1) whether the client posed near a cleaning station, (2) the time each client waited before being attended by the cleaner, (3) whether the client was in fact inspected or not, (4) inspection duration (with minimum duration considered as one second), and (5) the number of jolts by clients. Moreover, the frequency of chafing events against the substrate by each individual was registered.

3.2.4. Ectoparasite loads

Data previously collected for clients' ectoparasite loads (see Chapter 2) was used for the two selected reefs. However, individuals sampled for ectoparasite load assessment were not the same as those observed, and were only captured after all behavioural observations were finished. Additionally, the number of dark blemishes on the recorded ocean surgeonfish body surface was noted. The same was not possible to verify for longfin damselfish due to their dark colour.

3.2.5. Statistical analyses

Concerning the behavioural analysis, three behavioural correlates of service quality were measured: (1) clients' waiting time at cleaning stations, (2) inspection duration, and (3) client jolt rate, expressed as the number of jolts per 100 s of interaction time (Soares *et al.*, 2008b). Waiting time, inspections duration and jolt rate were averaged per individual. Since service quality depends on the existence of an interaction and/or posing behaviour, the sample size had to be reduced to the correspondent value. Non-parametric tests were used since the data did not meet the parametric assumptions, even after transformation. Mann-Whitney tests were performed for comparisons between the two reefs and between the two client species within each reef. All statistical analyses were two tailed, performed without outliers and conducted using IBM SPSS Statistics 22.

3.3. Results

3.3.1. Cleaners density

Considering cleaner fish overall density (bluehead wrasse and sharknose goby combined), and considering bluehead wrasse density alone, differences were found between reefs in both cases (Mann-Whitney, repetitively: $U_{10} = 4.50$, $P = 0.028$; $U_{10} = 1.00$, $P = 0.004$), and more cleaners were present in Water Factory. Sharknose goby density was similar between reefs (Mann-Whitney, $U_{10} = 14.50$, $P = 0.619$). Within each reef, cleaner species density differed from each other in Carmabi (the degraded reef) (Mann-Whitney, $U_{10} = 4.00$, $P = 0.026$), with a mean of 0.17 sharknose gobies m^{-2} and 0.48 bluehead wrasses m^{-2}, but in Water Factory (Water Factory) densities were similar, with a mean density for both cleaner species of 0.56 ind. m^{-2} (Mann-Whitney, $U_{10} = 17.00$, $P = 0.898$).

3.3.2. Parasite loads

Overall, on 62% of longfin damselfish and 100% of ocean surgeonfish captured, at least one ectoparasite was retrieved. Longfin damselfish had significantly higher ectoparasite load in Carmabi (Mann-Whitney, $U_{19} = 21.00$, $P = 0.016$), contrarily to ocean surgeonfish which did not differ in total ectoparasite loads (Mann-Whitney, $U_{18} = 39.00$, $P = 0.436$). However, within reef, ocean surgeonfish had more ectoparasites per centimetre than longfin damselfish, in Water Factory (Mann-Whitney, $U_{18} = 87.00$, $P = 0.004$), whereas in Carmabi ectoparasite loads were similar between client species (Mann-Whitney, $U_{21} = 50.00$, $P = 0.756$) (Figure 9).

Retrieved ectoparasites consisted in gnathiid isopods (Gnathiidae), caligid copepods (Caligidae) and capsalid monogeneans (Capsalidae) (Appendix I). Longfin damselfish only showed differences between reefs in capsalid loads (Mann-Whitney: gnathiids, $U_{17} = 64.00$, $P = 0.109$; caligids, $U_{17} = 60.00$, $P = 0.206$; capsalids, $U_{18} = 76.50$, $P = 0.038$), which were higher in Carmabi (Figure 9). Contrary, for ocean surgeonfish no ectoparasite family loads showed to differ between reefs (Mann-Whitney: gnathiids, $U_{18} = 59.50$, $P = 0.481$; caligids, $U_{18} = 45.00$, $P = 0.739$; capsalids, $U_{18} = 54.00$, $P = 0.258$) (Figure 9). As for differences between client species, these were significant only in Water Factory regarding caligids, with ocean surgeonfish harbouring more caligids per centimetre than longfin damselfish (Mann-Whitney: Carmabi, gnathiids, $U_{19} = 60.00$, $P = 0.756$; caligids, $U_{19} = 33.50$, $P = 0.132$; capsalids, $U_{18} = 68.50$, $P = 0.175$; Water Factory, gnathiids, $U_{16} = 20.00$, $P = 0.083$; caligids, $U_{16} = 4.00$, $P = 0.001$; capsalids, $U_{16} = 40.50$, $P < 0.050$) (Figure 9).
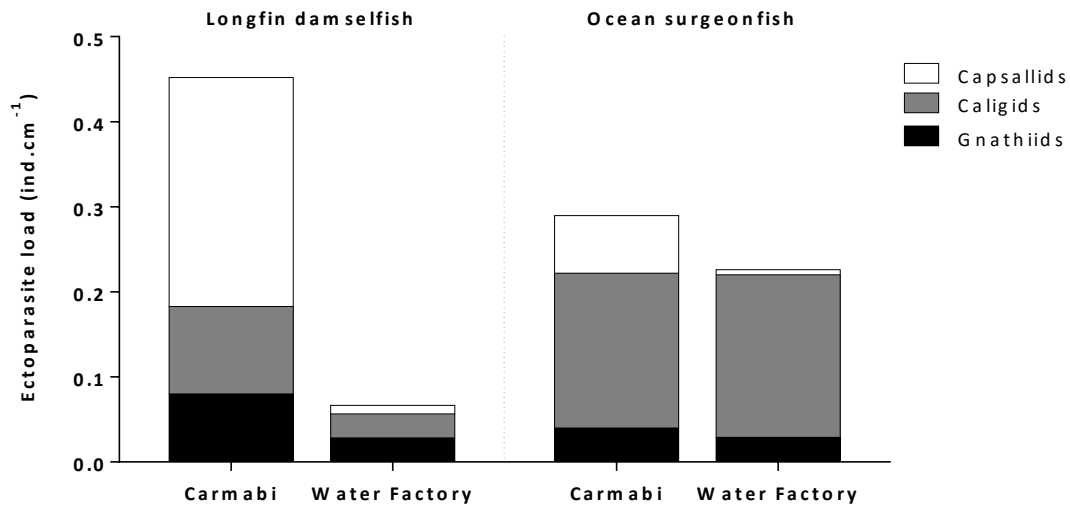
Figure 9 - Mean ectoparasite loads retrieved from both study client species in Carmabi (degraded reef) and Water Factory (less degraded reef). Bars are divided by ectoparasite families retrieved. Sample size: 11 longfin damselfish in Carmabi and 10 in Water Factory, and 10 ocean surgeonfish in each reef.

Additionally, ocean surgeonfish living in Carmabi had a higher number of dark blemishes (Appendix III) on their bodies when compared with those from Water Factory (Mann-Whitney, U_{17} = 13.50, P = 0.008) (Figure 10).
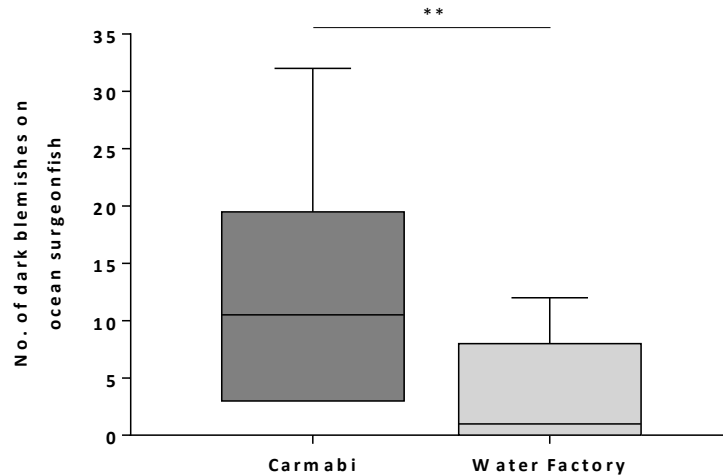
Figure 10 - Number of dark blemishes on ocean surgeonfish body and fins between Carmabi (degraded reef) and Water Factory (less degraded reef). Sample size: 10 ocean surgeons in Carmabi and 9 in Water Factory. Significant differences between reefs marked: ** Mann-Whitney, P < 0.010.

3.3.3. Cleaning behaviour

Focal clients were mostly observed interacting with sharknose gobies and juvenile bluehead wrasses (Appendix IV and V). Ten longfin damselfish had no cleaning stations inside their territory

(6 and 4 in Carmabi and Water Factory, respectively), nevertheless longfin damselfish were inspected by sharknose gobies in 31 of a total of 36 events, and by bluehead wrasses only on four events. Just once an interaction with a cleaner shrimp was observed, namely with a longfin damselfish. However, the shrimp species was not possible to identify due to bad visibility (possibly Pederson, *Ancylomenes pedersoni*, or spotted cleaner shrimp, *Periclimenes yucatanicus*). Ocean surgeonfish were inspected by sharknose gobies nine times and by bluehead wrasses eleven times, in a total of twenty events. However, not all focal clients interacted with cleaners during the observation time: nine out of 19 longfin damselfish (5 and 4 in Carmabi and Water Factory, respectively), and nine out of 21 ocean surgeonfish (4 and 5 in Carmabi and Water Factory, respectively) did not interact with cleaners (Table 4). Additionally, in Carmabi seven longfin damselfish and three ocean surgeonfish did not pose to any cleaner, and in Water Factory the respective number of individuals that were not observed posing was six longfin damselfish and five ocean surgeonfish (Table 4). Regarding chafing events, five longfin damselfish, in Carmabi, and eight longfin damselfish and three ocean surgeonfish, in Water Factory, did not chafe against the substrate (Table 4).

Table 4 - Summary of focal-follow observations of client species in the two reefs (Carmabi - degraded reef, and Water Factory - less degraded reef), including number of individuals observed, number of individuals with no chafing events, total number of chafing events, number of individuals that did not pose, total number of posing events, number of individuals with no client-cleaner interaction, total number of client-cleaner interactions.

| | Longfin damselfish | | Ocean surgeonfish | |
	Carmabi	Water Factory	Carmabi	Water Factory
Nr. ind. observed	10	9	10	11
Nr. ind. with no chafing events	5	8	0	3
Total no. of chafing events	9	2	48	19
Nr. ind. with no pose	7	6	3	5
Total no. of poses	12	14	13	9
Nr. ind. with no interactions	5	4	4	5
Total interaction number	19	17	14	7

Ocean surgeonfish chafed more frequently than longfin damselfish in both reefs (Mann-Whitney: Carmabi, $U_{18} = 88.50$, $P = 0.002$; Water Factory, $U_{17} = 76.00$, $P = 0.007$), but, between reefs, only ocean surgeonfish had higher chafing frequency, in Carmabi (Mann-Whitney, $U_{19} = 26.00$, $P = 0.043$), whereas longfin damselfish chafing was similar (Mann-Whitney, $U_{17} = 20.00$, $P = 0.083$) (Figure 11a). The number of poses in both reefs was similar for longfin damselfish (Mann-Whitney, $U_{19} = 58.50$, $P = 0.809$) and for ocean surgeonfish (Mann-Whitney, $U_{19} = 38.50$, $P = 0.251$). Pose frequency was also similar when comparing client species within reef (Mann-Whitney: more degraded reef, $U_{19} = 68.00$, $P = 0.387$; less degraded reef, $U_{19} = 53.00$, $P = 0.918$) (Figure 11b). Interaction frequency with cleaner organisms were similar between reefs for longfin damselfish (Mann-Whitney, $U_{17} = 46.50$, $P = 0.905$), and for ocean surgeonfish (Mann-Whitney, $U_{19} = 42.00$, $P = 0.387$), as it was similar between client species within each reef (Mann-Whitney: Carmabi, $U_{18} = 51.00$, $P > 0.050$; Water Factory, $U_{18} = 41.00$, $P = 0.552$) (Figure 11c).
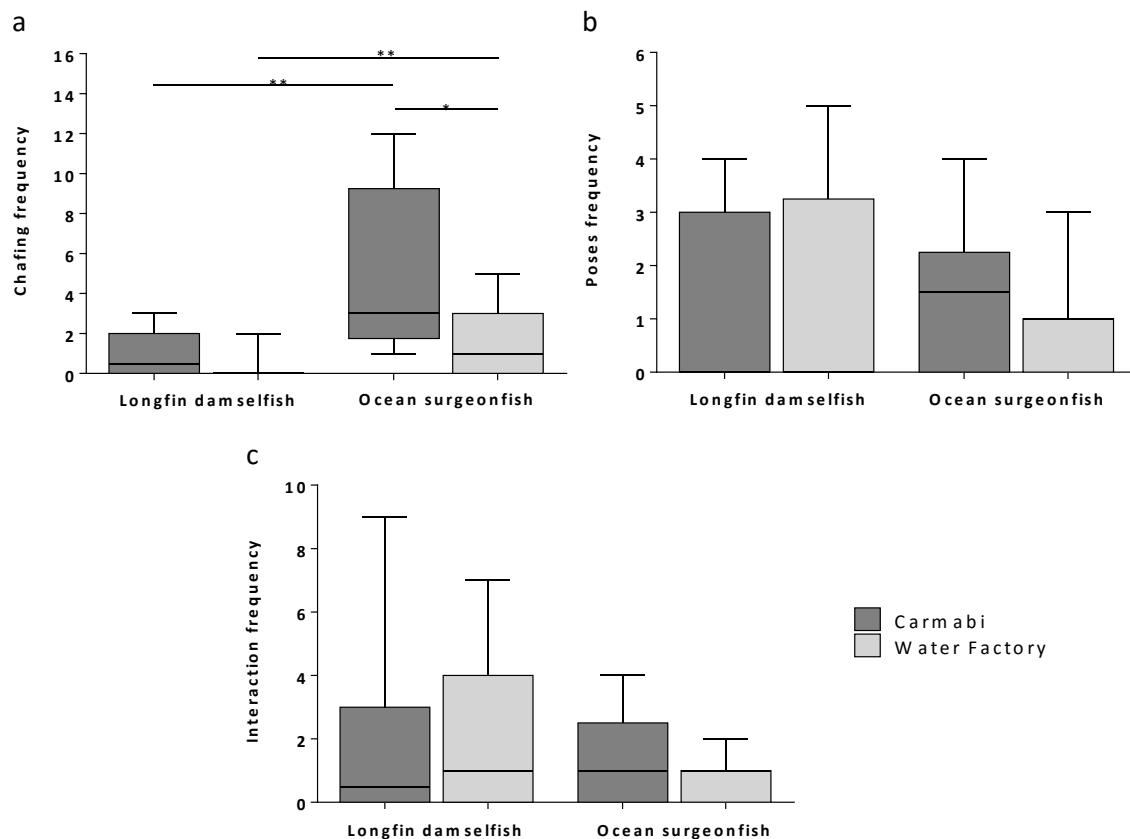
Figure 11 - Cleaning behaviours, a) chafing frequency, b) pose frequency, and c) interaction frequency with cleaners of longfin damselfish and ocean surgeonfish in Carmabi (degraded reef) and Water Factory (less degraded reef). Box-plots represent median, minimum and maximum, and 1st and 3rd quartiles. Sample sizes: longfin damselfish in Carmabi, $n_a = n_b = n_c = 10$, and in Water Factory, $n_a = n_b = n_c = 9$; ocean surgeonfish in Carmabi, $n_a = n_b = n_c = 10$, and in Water Factory, $n_a = n_b = n_c = 11$. Significant differences for client species between reefs and within reef between client species marked: * Mann-Whitney, P < 0.050; ** Mann-Whitney, P < 0.010.

3.3.4. Differences in service quality

Whenever longfin damselfish were observed posing at a cleaning station, the waiting time to be cleaned was similar in the two reefs (Mann-Whitney, $U_4 = 9.00$, $P = 0.100$), the same happened when ocean surgeonfish were considered (Mann-Whitney, $U_{10} = 11.50$, $P = 0.343$), and when the client species waiting time was compared within Water Factory (Mann-Whitney, $U_6 = 6.00$, $P = 0.786$). However, ocean surgeonfish waited more to be attended than longfin damselfish after posing in Carmabi (Mann-Whitney, $U_8 = 20.00$, $P = 0.033$) (Figure 12a).
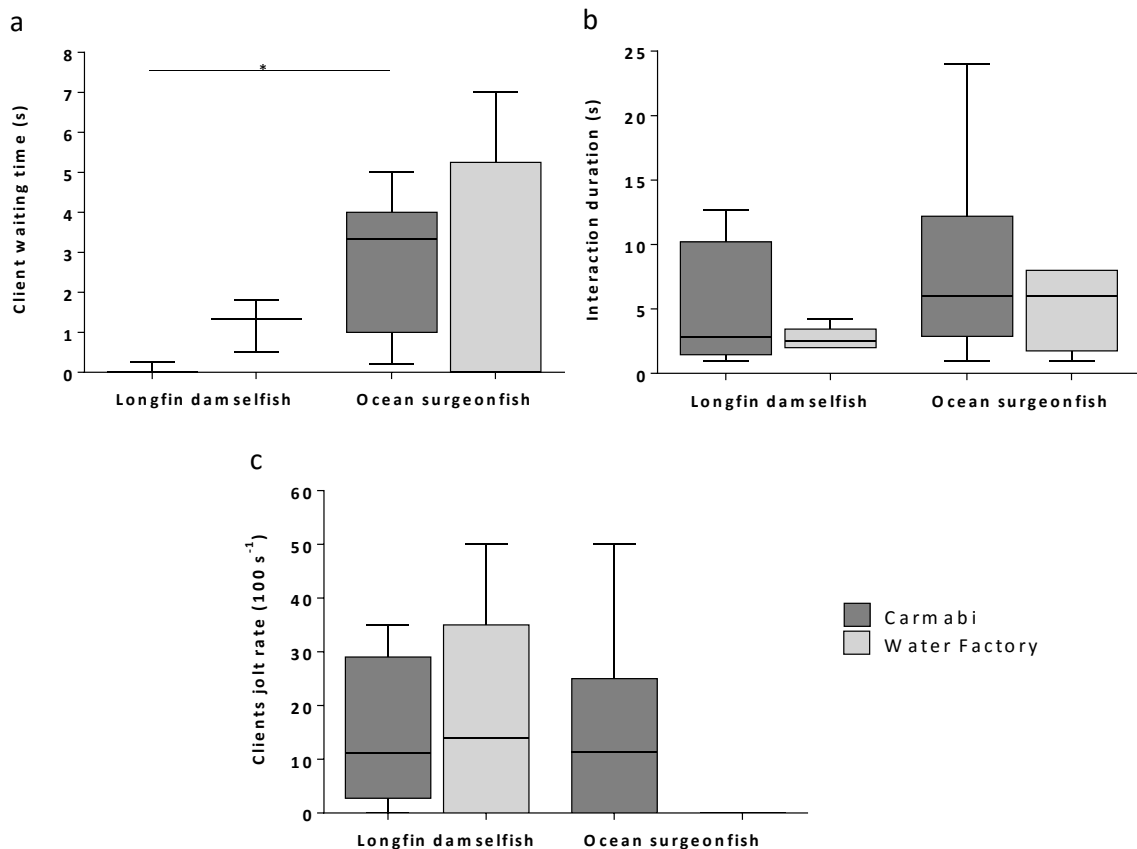
Figure 12 - Service quality measures: a) clients waiting time (s), b) interaction duration (s) and c) client jolt rate (100 s^{-1}) for longfin damselfish and ocean surgeonfish in Carmabi (degraded reef) and Water Factory (less degraded reef). Box-plots represent median, minimum and maximum, and 1st and 3rd quartile. Sample size: longfin damselfish in Carmabi, $n_a = 3$, $n_b = n_c = 4$, and in Water Factory, $n_a = 3$, $n_b = n_c = 5$; ocean surgeonfish in Carmabi, $n_a = 7$, $n_b = n_c = 6$, and in Water Factory, $n_a = 5$, $n_b = 6$, $n_c = 5$. Significant differences for client species between reefs and within reef between client species marked: * Mann-Whitney, P < 0.050.

Client-cleaner interaction duration was similar between reefs for both species (Mann-Whitney: longfin damselfish, $U_7 = 7.00$, $P = 0.556$; ocean surgeonfish, $U_9 = 15.50$, $P > 0.050$), as well as between client species within each reef (Mann-Whitney: Carmabi, $U_7 = 12.50$, $P = 0.556$; Water Factory, $U_9 = 21.00$, $P = 0.329$) (Figure 12b). Finally, client species jolt rates were also similar

between reefs (Mann-Whitney: longfin damselfish, $U_9 = 19.00$, $P > 0.050$; ocean surgeonfish, $U_9 = 5.00$, $P = 0.151$), and between species within reef (Mann-Whitney: Carmabi, $U_7 = 11.00$, $P > 0.050$; Water Factory, $U_9 = 5.00$, $P = 0.151$), even when compared with ocean surgeonfish that did not jolt in Water Factory (Figure 12c).

3.3.5. Parasite influence in cleaning behaviour

In Carmabi, ocean surgeonfish with more dark blemishes interacted more often with cleaners (Spearman correlation, interaction frequency, $r_s = 0.782$, n = 10, $P = 0.007$), but no other significant correlation was found between the frequency of blemishes and the cleaning measures analysed (Spearman correlation: chafing frequency, $r_s = -0.453$, n = 10, $P = 0.188$; pose frequency, $r_s = 0.370$, n = 10, $P = 0.292$; waiting time, $r_s = 0.234$, n =7, $P = 0.613$; interaction duration, $r_s = 0.600$, n = 5, $P = 0.285$; jolt rate, $r_s = 0.294$, n = 6, $P = 0.572$). In Water Factory no significant correlations between dark blemishes and all cleaning measures were found (Spearman correlation: interaction frequency, $r_s = -0.272$, n = 9, $P = 0.479$; chafing frequency, $r_s = -0.067$, n = 9, $P = 0.864$; pose frequency, $r_s = 0.523$, n = 9, $P = 0.148$; waiting time, $r_s = 0.949$, n =4, $P = 0.051$; interaction duration, $r_s = -0.410$, n = 5, $P = 0.493$; there were no jolts).

3.4. DISCUSSION

Although several factors may underlie clients' motivation to seek cleaning, ectoparasites are more often proposed as a main driver (Grutter, 2001; Cheney and Côté, 2003b; Sikkel *et al.*, 2004). This study shows that two important herbivore species in coral reefs appear to diverge in terms of vulnerability and react differently to parasite infection towards cleaning, showing that different patterns and motivations towards cleaning may depend on ectoparasites, but also on other parasite infections, such as dermal parasitation.

3.4.1. Longfin damselfish

Overall, only longfin damselfish ectoparasite loads varied significantly between reefs, and were higher in Carmabi (degraded reef), mostly due to capsalid monogenean loads. Carmabi was also the reef where fewer cleaner fish were found. As a result, less cleaning stations were available to clients, which, along with reef degradation, may contribute to an increase of clients' ectoparasite loads (Grutter, 1998; Sikkel *et al.*, 2000, 2009; Artim and Sikkel, 2013). Nevertheless, the increase of ectoparasite loads was not translated into significant behavioural shifts regarding cleaning motivation. Such results came as a surprise, as in other locations more parasitized longfin damselfish showed to pose to and visit cleaners more often and for longer periods of time (Grutter, 1995, 2001; Sikkel *et al.*, 2000, 2004; Arnal *et al.*, 2001; Côté and Molloy, 2003; Cheney

and Côté, 2005). However, most of these studies considered only gnathiids as a measure, which in the present study showed no difference between reefs.

Gnathiids are one of the most well studied ectoparasite families. Gnathiids are present in marine environments almost worldwide (Tanaka, 2007) and, along with caligids, are the most frequently found ectoparasites (Grutter and Poulin, 1998; Grutter, 2002). Gnathiids are the preferred ectoparasite ingested by cleaners (Losey, 1974; Grutter, 2002) and are vectors to other pathogens, such as blood parasites (Cook *et al.*, 2015), and they are capable of killing their host (Grutter *et al.*, 2011). However, other parasites may also share a relevant influence on species behaviour. For instance, capsalid monogeneans are also eaten by cleaners, both fish and shrimps (Grutter and Bshary, 2003; Becker and Grutter, 2004; McCammon *et al.*, 2010; Souza *et al.*, 2014), although they are harder to find in gut contents due to their soft, easily digested body (Becker and Grutter, 2004). Monogeneans are similarly capable of causing irreversible damages to hosts, facilitating secondary infections and being potentially lethal (Kanenko II *et al.*, 1988). In the present study, capsalid monogenean loads were found to be higher in Carmabi (the degraded reef). However, no cleaning behaviour differences were found to be significant.

Hence, two questions emerged. First, if different ectoparasites induce or influence cleaning behaviour in different ways. This could explain why no behavioural changes were observed where longfin damselfish had heavier capsalid loads, but similar gnathiid and caligid loads Surprisingly, no comparative studies on the matter were found. Second, if there is a threshold of irritability or health deterioration that must be reached in order to promote a necessity to be cleaned. In Grutter (2001) this same question arose, but if such threshold exists is still unknown. However, there might exist some clues pointing to this direction, as Soares *et al.* (2008b) found differences in cleaning behaviour only where client fish gnathiid loads were higher, and in Cheney and Côté (2003b) the authors advance the hypothesis that at low densities ectoparasites probably cause minimal deleterious effects, and that solely in places with more ectoparasite infections the benefits from visiting a cleaning station are greater.

In the case of longfin damselfish, territoriality is a crucial factor to be taken into account. Costs involved in visiting a cleaning station within territory boundaries are lower, since there is no need to leave the territory unguarded in order to seek cleaning. Therefore, as Cheney and Côté (2001; 2003b) found, longfin damselfish with a cleaning station within their territory were less infected with gnathiids, and were also more frequently cleaned. In the present study nine individuals did not have a cleaning station in the territory, which may have introduced confounding information. Additionally, because the main cleaning activity, together with heavily infected fish, occurs during

dawn and dusk (Chambers and Sikkel, 2002; Sikkel *et al.*, 2004, 2006) future studies should focus sampling efforts during the mentioned periods. Then, the effects on cleaning behaviour and associate benefits might be more pronounced or easily detected, due to a greater ectoparasite reduction (Cheney and Côté, 2003b).

3.4.2. Ocean surgeonfish

Ocean surgeonfish' ectoparasite loads were not significantly different between reefs. However, this species was still experiencing behavioural differences and a seemingly increase in dermal parasites (using dark blemishes as a proxy). In Carmabi, the degraded reef, ocean surgeonfish chafed more against the substrate (than longfin damselfish, and, than ocean surgeonfish from Water Factory) as a self-cleaning attempt, waited longer to be cleaned (than longfin damselfish), and those with more dark blemishes interacted more often with cleaners. This chain of results led to the conclusion that ocean surgeonfish seem to be a more vulnerable species and more willing to invest in cleaning events. Indeed, previous studies have reported high rates of infection by monogeneans, turbellarians, and parasitic copepods in Acanthuridae (Sikkel *et al.*, 2009; McCammon *et al.*, 2010; Bernal *et al.*, 2015; de Graaf and Simal, 2015), and they are known to be a quite sensitive fish family.

Ectoparasites are expected to be a stressful factor for their hosts (Ros *et al.*, 2011). Nevertheless, it is possible that the parasitism associated with the dark blemishes found on ocean surgeonfish could be more efficient in stress increase, being in turn capable of supressing the immune system and leaving infected fish more prone to other infections and diseases (Yada and Nakanishi, 2002). However, the full impact of dermal parasite infection remains unknown. Thus, further work on this topic should consider focusing its sampling effort on ocean surgeonfish from the most degraded reef (*i.e.* Carmabi), due to their apparent vulnerability.

3.4.3. Final remarks

This study shows that two important herbivorous species in coral reefs react differently to parasite infection towards cleaning. Ocean surgeonfish appeared to be more vulnerable than longfin damselfish, and more willing to invest in cleaning, either by chafing or by attending cleaning stations. Interestingly, underlying this investment seemed to be dermal parasites (with dark blemishes as proxy), even more than ectoparasite loads. However, a joint action of factors cannot be ruled out. Future work is clearly needed to further investigate how ectoparasites and dermal parasites are affecting fish's behaviour and health, and how reef degradation is contributing to it.

4. FINAL CONSIDERATIONS

Coral reefs are under increasing pressure and reef degradation has reached worrisome levels (Wilkinson, 2000; Gardner *et al.*, 2003; Pandolfi *et al.*, 2003). The crescent decrease in live coral allows the proliferation of some ectoparasites (Grutter, 1998; Sikkel *et al.*, 2000; Artim and Sikkel, 2013). A good way to face this significant rise in parasitation, is for host fish to increase their investment on cleaning activities (*e.g.* Arnal *et al.*, 2001; Cheney and Côté, 2001; Sikkel *et al.*, 2004). The present study focused on parasites by first trying to establish a link between parasite loads and loss of live coral (recognizing other factors with potential influence on parasite abundance, such as fish density) (Chapter 2), and finally linking host parasite loads to cleaning behaviour response (Chapter 3).

In Curaçao, degraded reefs appear to provide favourable conditions for benthic stage ectoparasite proliferation, i.e. gnathiids and capsalids, at least when longfin damselfish are considered. This seems particularly true in Carmabi, where low live coral cover (ca. 5%) is clearly outweighed by a high cover of dead coral, algae and sponges (ca. 75%), substrate favourable to benthic ectoparasites, and where the near canal seems to favour the input of nutrients and sediments into the reef, decreasing its water quality. Host density was also shown to affect ectoparasite loads (caligids loads in the longfin damselfish, and gnathiid loads in the ocean surgeonfish) through a dilution effect (Côté and Poulin, 1995). However, ectoparasite loads found in the two most different reefs (Carmabi and Water Factory) were not enough to significantly change longfin damselfish behaviour as clients. In turn, ocean surgeonfish invested more in cleaning behaviour and it seemed to be a more vulnerable species to reef degradation, since poor environmental conditions together with parasite infection, may turn out to have a deleterious influence to fish health, putative stress response increase and immunological shifts (Yada and Nakanishi, 2002).

In the past few decades several studies have contributed to the body of work regarding reef degradation - parasites - cleaning interactions (Grutter, 1998; Côté, 2000; Sikkel *et al.*, 2000, 2009; Cheney and Côté, 2003d, 2005), however there are still plenty of questions to be answered. This becomes especially relevant with the crescent impact of climatic change on reef ecosystems, which harbour most of the biodiversity and have an intrinsic value to human populations. Indeed, parasites are a naturally occurring component, and have an important role in the ecosystem, but when their numbers get outbalanced they may have devastating impacts, both on hosts and in the ecosystem as a whole (Hudson *et al.*, 2006). In 1980's, the Caribbean region suffered a mass mortality of long spined black sea urchins, *Diadema antillarum*, among the most important herbivore organisms. Although the causes of this mass mortality are unknown, a pathogen

infection of some kind seems to be the most probable cause (Bak *et al.*, 1984). After this event, algal cover increased dramatically (in some places up to 95%), and other herbivores were not able to occupy the sea urchin role in the ecosystem, due to overfishing (Bak *et al.*, 1984). This may come as a good example as to why herbivores should be monitored in coral reef. Thus, in addition to Pomacentridae and Acanthuridae, future works should also consider the Scaridae family, in order to achieve a wider perspective of the main herbivores of the Caribbean, especially focusing on more vulnerable species. As an improvement to the present study, future research should also increase sample size; consider using the same fish for ectoparasite assessment and behaviour observation, to have a more precise link between the two variables, as well as using the surface area instead of fish total length for ectoparasite load estimation; collecting data regarding reef abiotic variables, as these also affect the ectoparasites and the fish themselves (Landsberg *et al.*, 1998; Marcogliese, 2005; Sasal *et al.*, 2007); and collecting data regarding ectoparasite emergence from the reef directly.

Finally, parasites, specifically the ectoparasites and dermal parasites (through blemishes), and cleaning behaviour (as a way to understand how fish are reacting) may be used to monitor coral reef degradation, having the advantage to have a relatively low impact on the fish population, since there is no necessity to kill sampled individuals. Additionally, with the contribution of future studies regarding parasites and cleaning behaviour contribution to fishes' stress levels and immunological response, these same variables may also be used to monitor reef fish health, and thus contribute to reef conservation.

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6. APPENDICES

Appendix I - Example of ectoparasite found on fish, a) Gnathiidae, b) Caligidae, and c) Capsalidae.
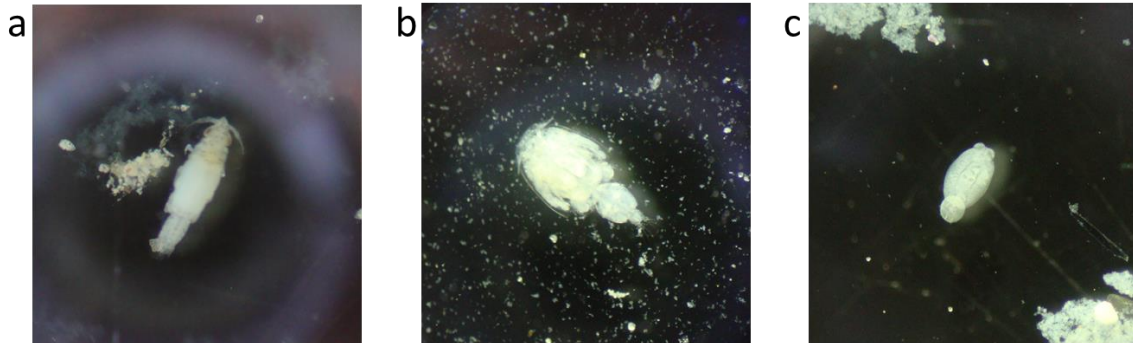
Appendix II - Visual comparison of benthic cover between (a, b) Carmabi, degraded reef, and (c, d) Water Factory, less degraded reef.
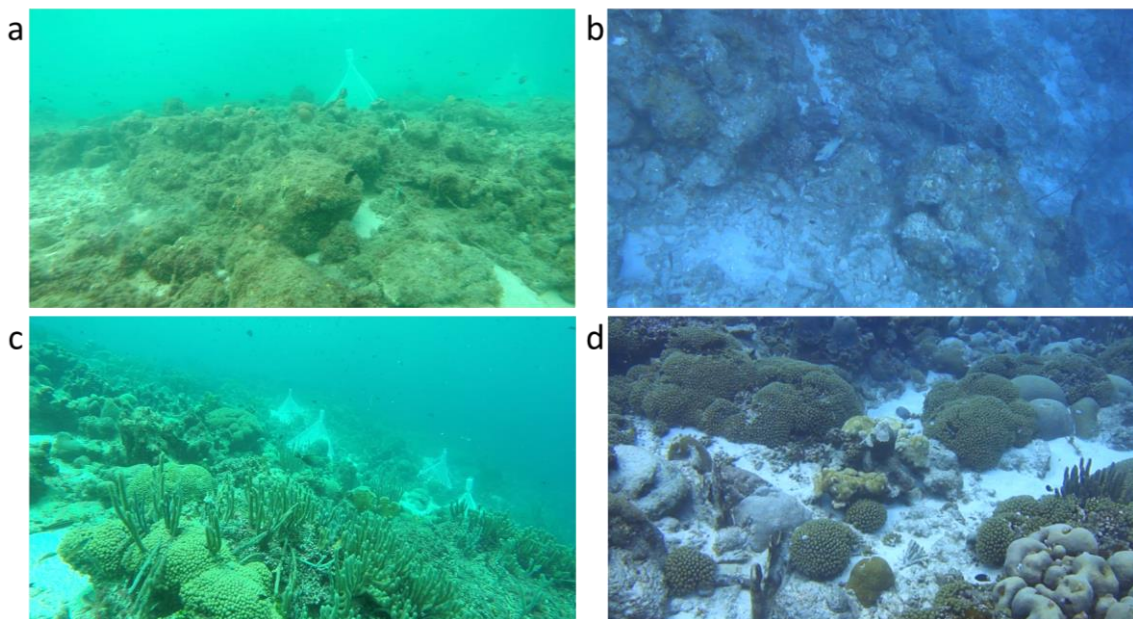
Appendix III - Dark blemishes (yellow arrows as example) on infected ocean surgeonfish (*Acanthurus tractus*)

Appendix IV - Cleaning interaction between longfin damselfish (*Stegastes diencaeus*) and sharknose goby (*Elacatinus evelynae*)

Appendix V - Cleaning interaction between ocean surgeonfish (*Acanthurus tractus*) and juvenile bluehead wrasses (*Thalassoma bifasciatum*)