

**The role of macroalgae
and the corallivorous fireworm *Hermodice carunculata*
on coral reef resilience in the Caribbean**



**Dissertation submitted by
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Dedicated to my parents and Sonni

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Thesis Abstract

This thesis is composed of five chapters representing studies that investigate the impact of two important stressors strongly influencing coral reef resilience in the Caribbean: macroalgal competition with corals and corallivory by fireworms. Macroalgae are superior competitors for space in coral reefs due to their 1) high ability to adapt to changing environmental conditions such as elevated nutrient availabilities and sea surface temperatures, 2) optimized reproductive strategies and life cycles, and 3) potent allelochemical characteristics during interaction processes. Invertebrate corallivory is an important stressor of present-day reefs, in which its impact can lead to fundamental changes in reef state. During a preliminary visit to Curaçao, Netherlands Antilles, the designated study area for this thesis, we observed high abundances of macroalgae and an invertebrate corallivore, the bearded fireworm *Hermodice carunculata*, particularly in the presence of macroalgae. Together with gastropods, fireworms constitute potentially the most influential corallivorous organisms in Caribbean reefs, and population outbreaks have been associated with impaired coral recovery after disturbances. Recent work showed that this invertebrate corallivore may also act as a vector for coral bleaching pathogens in the Mediterranean, but its importance with regard to coral reef resilience has not been addressed since the late 1960s.

Reciprocally, impaired coral reef resilience will inevitably lead to 1) declining reproductive output and recruitment, 2) higher susceptibility to and longer regeneration times after disturbances such as mass coral bleaching and disease events, and 3) the loss of diversity and structural integrity. Alternate and stable state macroalgae-dominated reefs deprived of original functionality are of major concern and have been observed throughout the Caribbean, especially under declining environmental conditions, which hold true for most Caribbean reef systems. The fringing reefs of Curaçao, Southern Caribbean, are considered relatively intact for Caribbean standards, i.e. still in a coral-dominated state, and therefore represent suitable study sites to carry out experimental work. Although detailed studies exist

with regard to macroalgal characteristics, very little is known about the corallivore *H. carunculata*, and no information exists with regard to their specific effects on scleractinian corals, particularly during coral-algal interactions, and their ecological role during impaired coral reef resilience scenarios.

The objectives of my thesis thus were to investigate separate and synergistic effects of these two prominent stressors on Caribbean reef systems. As a fundament, we provide the first comprehensive ecological study on principle characteristics of the corallivore *H. carunculata*. Using multiple approaches including in situ experiments, quantitative analytical tools and molecular techniques, we investigated its ecological role within coral reefs and determined its origin and distribution based on its phylogeny. Then, we explored the specific effects of macroalgal competition and corallivory on the major reef building coral in Curaçaoan reefs. Supplementary in situ and laboratory experimental studies provided detailed information on the kind, diversity and severity of their detrimental impacts for scleractinian coral communities. Ultimately, potentially synergistic effects of both stressors for Curaçao's major reef building coral were identified under natural conditions and discerned using specific laboratory experiments.

Chapter 1 of my thesis, "Distribution, habitat specificity and food preference of the corallivorous fireworm *Hermodice carunculata* in a typical Caribbean reef", provides basic yet essential knowledge on ecological features of fireworms in coral reefs. Although present in all reef areas, highest fireworm abundances were found along the coral-depauperate shoreline. The simulation of coral-algal interactions in coral-dominated reef areas resulted in the attraction of high fireworm abundances inside macroalgae in contact with live coral, assumedly due to available food sources in close proximity to a suitable refuge. Stable isotope analyses indicate an ontogenetic dietary shift in fireworms with increasing size, likely due to an expanded foraging range and food repertoire within the reef habitat. Furthermore, food choice experiments revealed decaying coral tissue as a preferred food source. These results identify *H. carunculata* as a spatially unconstrained,

omnivorous corallivore with a high dietary plasticity, implying potentially severe consequences for scleractinian corals in reefs with impaired resilience.

The experiments and quantitative analyses presented in this thesis were often based on *a priori* morphological identification of *H. carunculata* specimens. Molecular analysis, however, recently revealed *H. carunculata* to constitute of two different species, *H. carunculata* and *Hermodice nigrolineata*, the former being restricted to the Wider Caribbean, the latter ranging from the Mediterranean into adjacent Eastern Atlantic regions. Therefore, a molecular study was conducted in order to 1) reassure all ecological studies concern the same, distinct species, i.e. *H. carunculata*, 2) explore potentially cryptic fireworm species in the Southern Caribbean, and 3) complement the ecological baseline study on *H. carunculata* described in Chapter 1.

Within **Chapter 2**, “The curious case of *Hermodice carunculata*: an amphinomid polychaete exhibiting high population connectivity throughout the Atlantic Ocean”, we unravel the phylogeny of *Hermodice* sp. based on the analysis of several mitochondrial (COI and 16S) and nuclear (ITS) genes. The assumption that Curaçaoan reefs were dealing with morphologically indifferent, but genetically diverse, i.e. cryptic species, could be denied. This study reveals *H. carunculata* as widespread species with high population connectivity across the Caribbean and beyond. With regard to resilience patterns of Caribbean reefs, fireworm occurrences and corallivory may thus often be attributed to the species of *H. carunculata*. Furthermore, the results approve the morphological identification procedures conducted during each of my investigations.

Unlike *H. carunculata*, many Caribbean macroalgae and their species-specific characteristics are well described. However, investigations of different macroalgae during coral-algal interactions are scarce, although of great relevance. **Chapter 3**, “Contact with macroalgae causes variable coral mortality in *Montastraea faveolata*” demonstrates the variable, but consistently detrimental and rapid effects of different macroalgae on coral health during an in situ interaction experiment. A comparative aspect for the Wider Caribbean is provided by using the

major reef building coral species of most Caribbean reefs, *Montastraea faveolata*. Whereas the macroalgae *Lobophora variegata* and *Halimeda opuntia* slowly and gradually aggravated coral tissue condition, *Dictyota pinnatifida* and *Cladophora* sp. induced severe tissue deterioration within a few days after direct contact. The exudation of potent allelochemicals and especially the species-specific and unequal excretion of primary metabolites such as dissolved organic matter seem to be crucial in this context. Importantly, direct contact with macroalgae often resulted in coral mortality beyond the limited area of interaction, and we assume this disease-like response to be microbe-induced and driven. The data suggest that disease-like effects on scleractinian corals may occur irrespectively of macroalgal species, and that the susceptibility of corals to disease may increase after any form of prolonged coral-algal interaction. This study demonstrates the disastrous, specific effects of macroalgal competition and is followed by a respective study on the specific effects of corallivory on scleractinian corals.

Chapter 4, “Predation on coral settlers by the corallivorous fireworm *Hermodice carunculata*” shed light on the essential but unsolved question whether – and to what extent *H. carunculata* would predate on major reef building corals. We provide the first laboratory-based evidence of *H. carunculata* predated on coral recruits irrespectively of species investigated. Moreover, a size-related ontogenetic behavioral pattern for the corallivore was found in which small fireworms (< 2 cm length) caused 100 % recruit mortality, whereas the survival of recruits increased gradually to reach almost 100 % when exposed to large fireworms (> 6 cm length). Interestingly, no predation on healthy, adult corals regardless of coral species or fireworm size was recorded, suggesting a change, specifically a decrease in susceptibility of corals towards predation with increasing size. These findings are in line with and complement the observations of Chapter 1 and suggest that scleractinian corals may not be directly affected by *H. carunculata* predation once they overcome the critical juvenile life phase and as long as they remain healthy, i.e. display functioning defense mechanisms. This study highlights important and unobvious relationships between two organisms within the reef system. In order to

understand or foresee the response of complex ecosystems such as coral reefs to disturbance, it is essential to consider multiple stressors whenever possible. The relevance of these results are demonstrated in the following chapter, where healthy scleractinian corals are additionally manipulated by the presence of macroalgae.

Chapter 5, “Synergistic effects of algal overgrowth and corallivory on reef-building corals”, describes such an approach by simultaneously studying macroalgal competition and corallivory under natural and laboratory conditions. We examined the interaction among the calcareous green algae *Halimeda opuntia*, *Hermodice carunculata* and *M. faveolata*. The results of the separate effects confirm earlier observations described in Chapter 3 and 4. Strikingly, both factors were able to act in synergy to increase coral mortality, providing the first evidence of algal-induced corallivory. Although not confirmed for other combinations of macroalgae and corallivores and thus limited in wider applicability, such findings indicate the creation of positive feedback loops, whereby corallivory leads to increased macroalgal cover, which in turn leads to more algal-induced corallivory. Within this study, we demonstrate that coral reefs could become more prone to corallivory under high algal abundance and highlight the complex interactions between corallivores, algae and scleractinian corals on present-day reefs.

The objectives of my thesis derive from the urgency to advance scientific knowledge of major stressors of present-day reefs and their influence on coral reef resilience. Although these objectives were met, the outcome is rather discouraging when put into perspective with regard to coral reef resilience. The findings lend support to the assumption that the multitude of co-occurring effects, i.e. 1) increased recruitment bottlenecks due to corallivory on juvenile corals, 2) rapid coral deterioration caused by macroalgal contact, and 3) synergistic effects which increase coral mortality, may reduce coral reef resilience in the Caribbean. Consequently, scleractinian corals will not be able to suppress macroalgal proliferation in the long run, particularly after re-occurring natural disturbances. Nonetheless, the broad applicability of my findings will hopefully be useful in recognizing substantial

threats or changes in coral community structure and applying remediation measures to maintain coral reef resilience as much as possible.

Zusammenfassung

Diese Dissertation beinhaltet fünf Kapitel, welche Studien repräsentieren, die zwei wichtige Stressfaktoren, erstens das Auftreten von Makroalgen und deren Interaktion mit Steinkorallen und zweitens Korallivorie, und deren Effekte auf die Widerstandsfähigkeit von Korallenriffen untersuchen. Die Zielsetzung meiner Doktorarbeit war die Untersuchung und Ausleuchtung von spezifischen und synergistischen Effekten dieser beiden Stressfaktoren auf karibische Korallenriffe. Aufgrund der mangelnden Informationen über den zu betrachtenden Korallivor, *H. carunculata*, umfasste dies auch eine umfassende Studie über dessen Ökologie, in der seine Rolle innerhalb des Korallenriffsystems sowie seine phylogenetische Herkunft untersucht wurde. Weiterhin wurden die spezifischen Effekte der Makroalgen und des Korallivors auf die Steinkoralle *Montastraea faveolata*, die die wichtigste riffbildende Koralle in der Karibik darstellt, erforscht. In situ und laborbasierte Untersuchungen bieten detaillierte Information über die Art, Unterschiede und den Schweregrad der meist negativen Effekte. Abschließend wurden erstmals potentiell synergistische Effekte der beiden Stressfaktoren auf Curaçaos wichtigste, riffbildende Korallenart untersucht, und Erkenntnisse durch gezielte Laborexperimente verifiziert.

In **Kapitel 1** werden die fundamentalen ökologischen Eigenschaften der Feuerwürmer beschrieben und beleuchtet. Die höchsten Abundanzen an Würmern, obwohl in allen Riffzonen anzutreffen, wurden im Flachwasserbereich gefunden, der keinerlei Korallenbedeckung aufweist. Die Simulierung von Makroalgen-Korallen-Interaktionen in Korallen dominierten Riffzonen führte zur Anziehung großer Feuerwürmmengen innerhalb der Makroalgen, welche in Kontakt mit lebenden Steinkorallen waren. Grund hierfür ist wahrscheinlich die unmittelbare Kombination aus Schutzort und potentieller Nahrungsquelle. Stabile Isotopenanalysen zeigen ontogenetisch-erklärbare Veränderungen der Ernährung mit zunehmender Größe der Feuerwürmer, was auf einen erweiterten Bereich während der Nahrungssuche sowie des Nahrungsrepertoires bei größeren Individuen rückschließen lässt. Außerdem

enthüllte ein Fütterungsexperiment mit sehr unterschiedlichen Nahrungsquellen Steinkorallen mit degeneriertem Gewebe als bevorzugte Nahrungsquelle dieses Korallivors. *H. carunculata* ist ein räumlich anpassungsfähiger und in unterschiedlichen Habitaten anzutreffender Allesfresser mit hoher Plastizität bezüglich seines Nahrungsrepertoires. Dies kann zu erheblichen Konsequenzen für Steinkorallen in Riffen mit einer verminderten Widerstandsfähigkeit führen.

Die Revidierung der ursprünglichen taxonomischen Einteilung *H. carunculatas* in zwei unterschiedliche, aber morphologisch sehr ähnliche Arten legte eine molekulargenetische Studie nahe, welche 1) die Überprüfung, dass sich unsere ökologischen Studien ausschließlich mit der Art *H. carunculata* befassen, 2) die Identifizierung kryptischer Arten des karibischen Raumes, und 3) die Komplementierung der ökologischen Eigenschaften *H. carunculatas* (Kapitel 1) zum Ziel hatte. In **Kapitel 2** wird die Phylogenie, d.h. die Herkunft und Zusammengehörigkeit von *H. carunculata*, basierend auf der Analyse verschiedener mitochondrialer (COI und 16S) und nuklearer (IST) Gene, untersucht. Die Annahme, dass in den Korallenriffen Curaçaos morphologisch gleiche, aber genetisch unterschiedliche Feuerwurmart, mit eventuell unterschiedlichen Eigenschaften, vorkommen, wurde nicht bestätigt. Diese Studie identifiziert *H. carunculata* als weit verbreitete Art mit hoher Populationskonnektivität im karibischen Raum und darüber hinaus. In Bezug auf die Widerstandsfähigkeit karibischer Korallenriffe ist eine Zuweisung beobachteter Feuerwurmabundanzen und damit eventuell assoziierter Korallivorie zu der Art *H. carunculata* als sinnvoll anzusehen.

In **Kapitel 3** werden die variablen und schnell auftretenden Effekte unterschiedlicher Makroalgen auf die Gesundheit von Steinkorallen anhand eines in situ Interaktionsexperiments aufgezeigt. Durch das Bezugnehmen auf die wichtige, riffbildende Steinkoralle *M. faveolata* wird ein komparativer Aspekt für das karibische Gebiet ermöglicht. Während die Makroalgen *Lobophora variegata* und *Halimeda opuntia* langsam aber stetig den Gesundheitszustand des Korallengewebes beeinträchtigten, führten *Dictyota pinnatifida* und *Cladophora* sp. zu einer extrem

schnellen und gravierenden Zerstörung des Gewebes innerhalb von nur wenigen Tagen. Die Ausschüttung potenter allelochemischer Stoffe, eventuell auch zur eigenen Verteidigung, und besonders die artspezifische und in ihrer Menge unterschiedliche Exkretion primärer metabolischer Stoffe wie z.B. gelöstes, organisches Material, spielen in diesem Zusammenhang vermeintlich eine sehr wichtige Rolle. Es ist wichtig zu betonen, dass der direkte Kontakt zu Makroalgen oftmals zu absterbendem Korallengewebe weit über die Grenze des direkten Kontakts hinaus führte. Wir vermuten, dass diese krankheitsähnlichen Symptome mikrobiell induziert und gesteuert werden. Die Ergebnisse weisen darauf hin, dass krankheitsähnliche Symptome bei Steinkorallen unabhängig von der Algenart auftreten und dass die Krankheitsanfälligkeit für Steinkorallen nach jedwedem verlängerten Makroalgenkontakt ansteigt. Diese Studie hat einen oftmals desaströsen Effekt von Makroalgen in direkter Konkurrenz mit Steinkorallen aufgezeigt. Als Folgestudie werden nun die spezifischen Effekte des Korallivors *H. carunculata* bezüglich der gleichen Korallenart vorgestellt.

Kapitel 4 erläutert die essentielle aber bisher unbeantwortete Frage, ob und in welchem Maß, *H. carunculata* an wichtigen Korallenarten frisst. Wir erbringen, wenn auch unter Laborbedingungen, den Beweis, dass *H. carunculata* junge Korallenrekruten - unabhängig von ihrer Art - frisst. Zusätzlich wurde gezeigt, dass es sich um ein Feuerwurmgrößen abhängiges Phänomen handelt, somit also eine ontogenetische Verhaltensweise darstellt. Während kleine Feuerwürmer (< 2 cm Länge) eine hundertprozentige Mortalität der Rekruten verursachten, stieg deren Überlebenschance graduell auf hundert Prozent an, wenn sie ausschließlich großen Feuerwürmern (> 6 cm Länge) ausgesetzt wurden. Überraschenderweise wird auch eine Veränderung der Anfälligkeit von Steinkorallen bezüglich des gefressen-Werdens aufgedeckt, da wir keinerlei Hinweise auf Fressvorgänge für gesunde, ältere Steinkorallen beobachten konnten - unabhängig von Korallenart und Feuerwurmgröße. Diese Resultate bestätigen und ergänzen unsere Beobachtungen aus Kapitel 1 und suggerieren, dass Steinkorallen nicht durch den Korallivor *H. carunculata* beeinträchtigt werden, sobald sie eine gewisse Größe erreichen bzw.

solange sie gesund bleiben. Die Relevanz dieser Studie wird im nächsten Kapitel sichtbar, indem der Gesundheitszustand von Steinkorallen durch die Interaktion mit Makroalgen zusätzlich beeinflusst wird.

Kapitel 5 beschreibt eine experimentelle Vorgehensweise, bei der die Interaktion zwischen Makroalgen und Steinkorallen und die Auswirkungen von Korallivorie gleichzeitig unter natürlichen und Laborbedingungen untersucht wurden; hier anhand der Makroalge *H. opuntia*, des Korallivors *H. carunculata* und der Steinkoralle *M. faveolata*. Beeindruckenderweise konnten wir im Falle simultan anwesender Stressfaktoren einen synergistischen Effekt feststellen, der zu einem erhöhten Korallensterben führte und den ersten Beweis für eine durch Makroalgen induzierbare Korallivorie liefert. Obwohl diese Synergie nicht für verschiedene Korallivoren und Makroalgen bewiesen wurde und allgemeine Rückschlüsse aus diesem Grund nur begrenzt geschlossen werden sollten, suggerieren die Ergebnisse einen positiven Rückkopplungseffekt: Makroalgen-induzierte Korallivorie führt zu einer erhöhten Makroalgenbedeckung, was wiederum zu einer gesteigerten Korallivorie führt. Diese Vermutung ist naheliegend und verdeutlicht die komplexen Interaktionen zwischen Korallivoren, Makroalgen und Steinkorallen in Korallenriffen.

Die Zielsetzung dieser Dissertation ergab sich aus der Dringlichkeit, den wissenschaftlichen Erkenntnisstand bezüglich der wichtigsten Stressfaktoren für heutige Korallenriffe und deren Widerstandsfähigkeit zu erweitern. Die Zielvorgaben, sowohl individuelle als auch synergistische Effekte dieser Stressfaktoren zu identifizieren und / oder zu quantifizieren, wurden erfüllt. Die Auswirkungen auf die Widerstandsfähigkeit von Korallenriffen sind als kritisch einzuschätzen. Die gesammelten Erkenntnisse deuten darauf hin, dass vor allem die Vielzahl gleichzeitiger Effekte, nämlich 1) die verminderte Regenerationsfähigkeit riffbildender Korallen durch gezielte Korallivorie an Korallenrekruuten, 2) die extrem schnellen und gravierenden Folgen des direkten Kontaktes mit Makroalgen und 3) der Synergismus beider Stressfaktoren, die Widerstandsfähigkeit von Korallenriffen stark beeinträchtigen können. Steinkorallen werden deshalb, insbesondere nach

immer wiederkehrenden Belastungen des Riffsystems, die Ausbreitung von Makroalgen nicht verhindern können. Nichtsdestotrotz wird sich die Übertragbarkeit dieser Ergebnisse hoffentlich als nützlich erweisen, Bedrohungen und Veränderungen in Korallenriffsystemen zu erkennen und falls möglich entgegen zu wirken, um die Widerstandsfähigkeit der Korallenriffe soweit es möglich ist zu erhalten.

Publication contributions

This thesis is based on the scientific manuscripts listed below. The specific contributions of each of the authors with regard to study idea and conceptualization, data acquisition, data analysis as well as manuscript completion are explained below.

Publication 1 (Chapter 1)

Alexander Wolf, Maggy Nugues, Christian Wild

(In preparation)

*Distribution, habitat specificity and food preference of the corallivorous fireworm *Hermodice carunculata* in a typical Caribbean reef.*

Contributions: The publication was initiated by A. Wolf and C. Wild. The study conceptualization was developed by A. Wolf and C. Wild. Data sampling was conducted by A. Wolf. Data analysis was carried out by A. Wolf. The manuscript has been written by A. Wolf with improvements by M. Nugues and C. Wild.

Publication 2 (Chapter 2)

Joseph B. Ahrens, Elizabeth Borda, Rômulo Barroso, Alexandra M.

Campbell, **Alexander Wolf**, Maggy Nugues, Anja Schulze

(Submitted for publication in *Molecular Ecology*)

*The curious case of *Hermodice carunculata*: an amphinomid polychaete exhibiting high population connectivity throughout the Atlantic Ocean*

Contributions: The publication is co-authored by A. Wolf and M. Nugues. The collaboration was initiated by A. Wolf and A. Schulze. Data sampling was conducted by A. Wolf. Data analysis was carried out by A. Wolf and continued by J. Ahrens. The manuscript has been written by J. Ahrens with improvements by the co-authors.

Publication 3 (Chapter 3)

Alexander Wolf, Christian Wild, Maggy Nugues

(Proceedings of the 12th International Coral Reef Symposium)

*Contact with macroalgae causes variable coral mortality in *Montastraea faveolata**

Contributions: The publication was initiated by A. Wolf and C. Wild. The planning of the study was developed by A. Wolf and C. Wild. Data sampling was conducted by A. Wolf. Data analysis was carried out by A. Wolf. The manuscript has been written by A. Wolf with improvements by C. Wild and M. Nugues.

Publication 4 (Chapter 4)

Alexander Wolf, Maggy Nugues

*(Accepted for publication in *Coral Reefs*)*

*Predation on coral settlers by the corallivorous fireworm *Hermodice carunculata**

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Publication 5 (Chapter 5)

Alexander Wolf, Maggy Nugues

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Synergistic effects of algal overgrowth and corallivory on reef-building corals

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Distribution, habitat specificity and food preference of the corallivorous fireworm *Hermodice carunculata* in a typical Caribbean reef

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This manuscript is in preparation for submission to *Coral Reefs*

Abstract

The corallivorous fireworm *Hermodice carunculata* can act as vector of coral bleaching pathogens and causes high coral mortality in combination with macroalgae. Despite these alarming findings, no ecological information about its distribution, habitat specificity and food preference is available. Our study addresses these features through a series of interconnected *in situ* experiments in a typical patch reef of Curaçao, Southern Caribbean. By deploying micropredator attracting devices (MAD's; i.e. macroalgal clumps in accessible mesh nets) along a water depth gradient to attract the fireworms, we found highest abundances (4.6 ± 0.8 individuals per 200 cm^2 algal cover) in the shallowest reef area of less than 1 m water depth, compared to very low numbers in the other reef areas (0.3 ± 0.2 to 0.9 ± 0.6 individuals per 200 cm^2 algal cover). *H. carunculata* abundances were not significantly different between different macroalgae (*Halimeda opuntia*, *Dictyota pinnatifida*, *Lobophora variegata*, *Cladophora* sp., and a plastic algal mimic as reference) that were used as microhabitat. However, significantly ($P < 0.0001$) more fireworms were attracted by MADs that were deployed on living scleractinian corals compared to rocky substratum, highlighting macroalgae as habitat for fireworms in the context of coral-algal interaction. In line with these observations, a food choice experiment revealed that fireworms were significantly more attracted by fresh organic material such as fish and decaying coral fragments than by healthy coral fragments, hydrozoans or gorgonians. Stable isotope analysis demonstrated a positive correlation of fireworm $\delta^{15}\text{N}$ values (6.6 ± 0.2 to 9.2 ± 0.4 ‰) with increasing length of the fireworms, suggesting an ontogenetic dietary shift. High fireworm abundances present in very organic matter-poor, sandy sediments at the shoreline suggest sediment-derived food sources such as meiofauna or mechanically disintegrated organic matter to play an important role for this omnivorous corallivore and putative deposit feeder. Our study provides insight into the distribution and dietary pattern of *H. carunculata* in Caribbean coral reef environments. Although we assume healthy corals to only marginally contribute to their diet, degrading corals appear to be a preferred food source for *H. carunculata*. These findings highlight the relevance of this corallivore under declining reef conditions, notably during coral-algal interactions, since they facilitate reef degradation and reduce resilience pattern.

Introduction

Invertebrate corallivores are able to inflict variable damage to scleractinian corals on a scale from minor and reversible injury of coral tissue to fundamental changes in reef state (Knowlton et al. 1990; Rotjan and Lewis 2008; Stella et al. 2011). Large-scale detrimental effects on community composition have been caused by the crown-of-thorns starfish *Acanthaster planci* (Birkeland and Lucas 1990; Chesher 1969; Pratchett et al. 2009) and muricid gastropods of the genera *Drupella* and *Coralliophila* (Brawley and Adey 1982; Morton et al. 2002; Turner 1994). Furthermore, other corallivores such as the nudibranch *Phestilla* sp. and the bearded fireworm *Hermodice carunculata* have been implicated in transmitting or increasing vulnerability to coral disease or coral bleaching, respectively (Dalton and Godwin 2006; Sussman et al. 2003; Williams and Miller 2005). The importance of invertebrate corallivory has often been discussed in the context of population outbreaks after major natural or anthropogenic disturbances (Baums et al. 2003a; Colgan 1987; Dulvy et al. 2004; Fabricius et al. 2010; Knowlton et al. 1990; Morton and Blackmore 2009). However, continuous and unnoticed effects of natural corallivore populations may play an important role in influencing coral community structure. This is supported by Pratchett (2005), who found *A. planci* to cause severe damage concomitant to a low increase in population size. In this respect, ecological information on the distribution, habitat selection and feeding habits of corallivores are necessary to understand their influence during dynamic shifts in coral community structure.

Habitat selection is important throughout various life stages of marine invertebrates (Meadows and Campbell 1972; Butman 1987; Werner and Giliam 1984). Biotic factors such as shelter, refuge against predators and food availability can determine habitat choices and thus distribution during larval and juvenile life phases. Gosselin and Chia (1995) described the macroalgae *Cladophora columbiana*, which provides refuge from predators and abundant food sources, as preferred microhabitat for newly hatched *Nucella emarginata* prosobranchs.

Corallivore distribution patterns are further influenced by habitat-specific abiotic characteristics such as water depth, reef exposure, and flow regimes (Cumming 1999; Reyes-Bonilla and Calderon-Aguilera 1999). Habitat complexity can increase species' densities, as has been shown for marine fish assemblages (Gratwicke and Speight 2005), intertidal gastropods (Beck 2000), epifaunal crustaceans in tropical seagrass meadows (Stoner and Lewis 1985) and many coral-associated invertebrates (Stella et al. 2011). Ontogenetic changes in habitat, e.g. triggered by interspecific competition or lower susceptibility to predation, are common among fish species such as bluegills and surfperches (Mittelbach 1981; Holbrook and Schmitt 1992), but also occur for the rock shrimp *Rhynchocinetes typus* (Ory et al. 2012), the seagrass-associated gastropod *Strombus gigas* (Ray and Stoner 1995), and muricids such as *Drupella cornus*, which move from very protective branching corals to less protective branching or massive *Porites* colonies with increasing size (McClanahan 1997). This behavioral response can also occur concomitantly with an ontogenetic shift in prey choice, a common phenomenon among many size-structured marine organisms (Cole 2010; Olson et al. 1995; Werner and Gilliam 1984).

Most corallivores, obligate and facultative, are considered specialists since they prefer certain scleractinian genera and avoid most other genera (Rotjan and Lewis 2008). *A. planici*, being a facultative corallivore, as well as the obligate muricids *Drupella* and *Coralliophila* sp. and the vermetid *Dendropoma* sp. preferentially consume acroporid, montiporid and pocilloporid coral species (Baums et al. 2003b; Morton et al. 2002; Pratchett 2007; Schoepf et al. 2010; Shima et al. 2010). However, they also display a high degree of dietary plasticity, which may allow their populations to persist after even radical changes in prey community composition (Hayes 1990; Carroll and Wethey 1990). Optimal foraging theory would predict that coral-feeding organisms choose prey that maximize energetic return (Ormond et al. 1976) and thereby selectively consume prey with the highest calorific content (Keesing 1990), carbon-to-nitrogen ratio (Graham 2007) or high carbohydrate, protein or lipid content (Stella et al. 2011; and references therein). The fireworm *H. carunculata* extends its prey choice from various coral species,

preferably acroporid species, to gorgonians, hydrozoans and anthozoans (Ott and Lewis 1972; Lewis and Crooks 1996; Vreeland and Lasker 1989; Witman 1988). Interestingly, Jumars (1993) discussed the possibility of polychaetes to restrict themselves to detritivory, if the occupied habitat favors this feeding mode. Since *H. carunculata* is often observed in the shallowest, coral-depauperate reef zones, this facultative corallivore may display an even greater feeding plasticity than other corallivores.

Recently, *H. carunculata* has been shown to cause high coral mortality in interaction with macroalgae (Wolf and Nugues, in review). Although being widespread and abundant in tropical and temperate seas and its corallivorous character has long been described (Marsden 1962), no recent information about its distribution, habitat specificity and food preference is available. Through a series of interconnected *in situ* experiments in a typical Caribbean patch reef, we investigated i) its occurrence along a depth gradient and microhabitat-related preferences by offering different macroalgae, ii) its foraging efficiency and food preferences, and iii), its dietary patterns using stable isotope analysis. We provide experimental evidence for a strong variability in occurrence of *H. carunculata* between different reef habitats, reveal an ontogenetic shift in its diet, and discuss underlying reasons and consequences of these findings in a broader perspective. Such baseline information on the abundances of well-known, but understudied corallivores are essential for recognizing shifts in community structure on present-day coral reefs.

Material & Methods

Experimental setup

All experimental work was conducted at “Buoy Zero” (69°58’ 26” N, 12°07’ 27”W), a typical patch reef on Curaçao, Southern Caribbean, between September and December 2011. The sampling of fireworms, their potential food sources, and sediments for subsequent stable isotope analysis was conducted at the experimental

site Buoy Zero and partly in the close-by channel leading into Buoy Zero Bay. *H. carunculata* is a very cryptic invertebrate on coral reefs, and abundance data are difficult to obtain. Therefore, we used an indirect mean of attracting the corallivores by deploying small mesh nets (mesh size 1 cm) filled with different macroalgae according to Wolf and Nugues (in review).

The *in situ* experiments comprised various series of net deployments in order to i) estimate fireworm abundances along a depth gradient (Exp. 1a), ii) evaluate preferences of the fireworm for different macroalgae as microhabitat (Exp. 1b), and iii) determine its foraging efficiency and aggregation behavior depending on the food sources enclosed within the algal nets (Exp. 2a, b). After each experiment ended, the nets including all epifauna were collected with ziplock bags. All fireworm specimens were identified according to Fauchald (1979) and Yáñez-Rivera et al. (2009), measured and partly prepared for stable isotope analysis. Fireworm densities will be expressed as number of individuals per 200 cm² algal surface area.

Experiment 1a) Fireworm abundances along a depth gradient

Mesh nets were deployed along a depth gradient comprising different reef zones (n = 7 replicates): 1) < 1m water depth (Shoreline); 2) 2 - 3 m (Shallow Reef Rock); 3) 3 - 5 m (Shallow Reef Sand); 4) 6 - 12 m (Drop Off); 5) 14 - 16 m (Deep Reef). The algal nets were collected after 21 days exposure on the reef; worms were treated as described above. Benthic functional groups surrounding each net were recorded using a PVC quadrat (4 m diameter, n_{total} = 35). Relative coverage and species of scleractinian corals and macroalgae were recorded, along with percentages of crustose coralline algae, turf assemblages (< 0.5 cm canopy height), and bare substratum (henceforth mentioned as CTB, according to Aronson and Precht 2000), sand, cyanobacterial mats, rubble, sponges around the net. Reef rugosity (expressed as rugosity index R. I.) was measured using the chain method; see McCormick 1994).

Experiment 1b) Fireworm abundances in different macroalgae as microhabitat

Four different macroalgae, common on Caribbean coral reefs, and 1 artificial algal mimic were used as net fillings (n = 7 replicates): 1) *H. opuntia*; 2) *Dictyota pinnatifida*; 3) *Cladophora* spp.; 4) *Lobophora variegata* and 5) Plastic algal mimic. All nets were deployed in the drop off area (6 - 10 m water depth) on two different underlying substrates, i.e. rock and live coral tissue (*M. faveolata*). Nets for the latter were fixed with nails and rubber bands on adjacent rock substratum without damaging the underlying coral tissue. The experiment ended after 21 d. Again, benthic functional groups were recorded via 4 m quadrats around each net (n = 70).

Experiment 2a) Foraging efficiency of H. carunculata

Small pieces (3 cm²) of fresh organic material (Red snapper; *Lutjanus campechanus*) were enclosed in nets filled with *H. opuntia*, deployed in the drop off area (6 - 10 m water depth), and left for 6, 12, 24, 48, and 96 h, respectively (n = 7 replicates). Stepwise net retrieval and fireworm count determined the optimum time span for the subsequent fireworm attraction experiment.

Experiment 2b) H. carunculata attraction to different food sources

Small pieces (3 cm²) of 6 different food sources were enclosed in nets filled with *H. opuntia* (n = 7 replicates): 1) Control treatment; no food source enclosed; 2) crab substitute meat; 3) the hydrozoan *Millepora complanata*, 4) the gorgonian *Pseudoplexaurus* spp.; 5) healthy fragments of *M. faveolata*; 6) fresh organic material (*L. campechanus*); 7) decaying fragments of *M. faveolata*. All nets were retrieved after 48 h and fireworm numbers counted. This period ascertained a high amount of attracted fireworm specimens (based on results of Exp. 2a), without the food source being depleted and fireworm specimens having disappeared.

Stable isotope and GC analysis

All samples of fireworms, potential food sources, and sediment probes, were dried at 40 °C for 3 d and stored for further analysis. Samples were ground to a homogenous

powder and analyzed for total carbon (C_{tot}) and nitrogen (N_{tot}) content by high-temperature combustion in an elemental analyzer (EuroVector EURO EA 3000). Fireworms possess calcareous bristles, i.e. both the organic and inorganic carbon percentages were measured. C_{org} and $\delta^{13}\text{C}$ samples were pre-treated with 1 N HCl and dried at 40 °C before analysis. Stable isotope compositions ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) were analyzed in a gas isotope ratio mass spectrometer (Thermo Finnigan, Delta Plus) after high-temperature combustion in an elemental analyzer (Thermo Finnigan, Flash EA 1112). The $\delta^{15}\text{N}$ values are given as ‰ deviation from the nitrogen isotope composition of atmospheric air and $\delta^{13}\text{C}$ as ‰ deviation from the carbon isotope composition of the standard (PDB carbonate).

Statistical analysis

Statistical analyses were conducted with the programs STATISTICA (Windows, release 9.0), PRIMER (Primer-E Ltd., release 6.1.11) and Sigmaplot (Windows, release 12.2). Results were regarded as statistically significant at $p = 0.05$, unless stated differently. Differences between treatments for the experiments 1a, 2a and 2b were assessed by non parametric Kruskal-Wallis by Ranks analysis. The individual differences among treatments were evaluated by Kruskal-Wallis multiple comparison Z value tests with Bonferroni adjustment. For experiment 2b, a Scheirer-Ray-Hare test (non-parametric equivalent of 2-way ANOVA) was applied to test for significant differences of macroalgal treatments, substrate types, and their interaction (Dytham 1999). Spearman's Rank analyses assessed correlations between highest fireworm abundance and the dominant functional group coverage along the experimental depth gradient while data was rank transformed prior to analysis. Pearson's moment-product correlation was applied to assess interactions between stable isotope composition and organic matter content of the sediment.

Results

Fireworm abundance in relation to water depth, macroalgae as microhabitat and benthic community structure

The highest fireworm abundances occurred on the shoreline (< 1 m water depth) with Means of 4.6 ± 0.8 individuals per 200 cm² algal cover. The abundances in this reef zone differed significantly from all abundances at other water depths, i.e. reef zones (Kruskal-Wallis ANOVA on ranks, $H = 17.5$, $df = 4$, $P = 0.002$; for multiple comparisons see Appendix 1) (Fig. 1a). Fireworm numbers along the water depth gradient from 2 - 16 m were consistently very low (0.3 ± 0.2 to 0.9 ± 0.6 individuals per 200 cm² algal surface area).

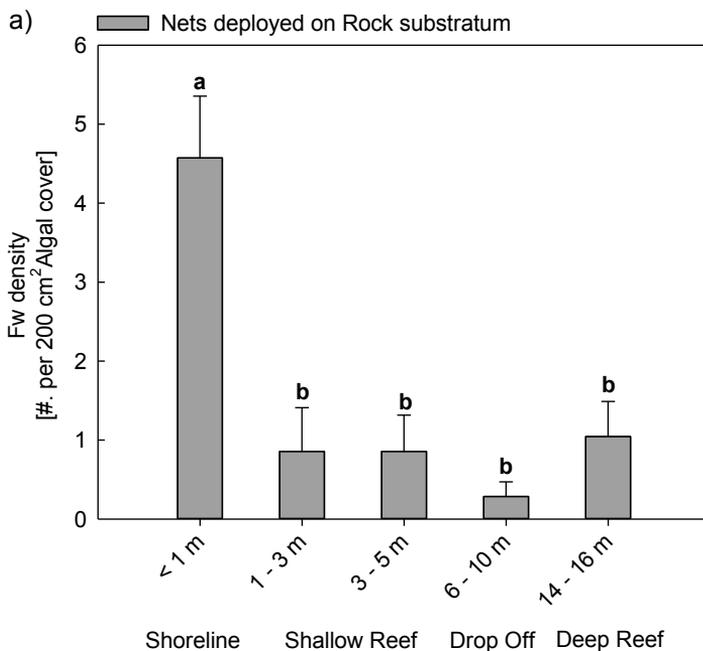


Fig. 1a) *Hermodice carunculata* abundances found in mesh nets filled with *Halimeda opuntia* and deployed along a depth gradient at the experimental site Buoy Zero (Means \pm SE, $n = 7$). Nets were fixed on rock as underlying substrate whenever possible. Data on fireworm abundances were analyzed by Kruskal-Wallis ANOVA on Ranks. Letters indicate homogeneous subgroups by multiple comparison z value tests (a - b).

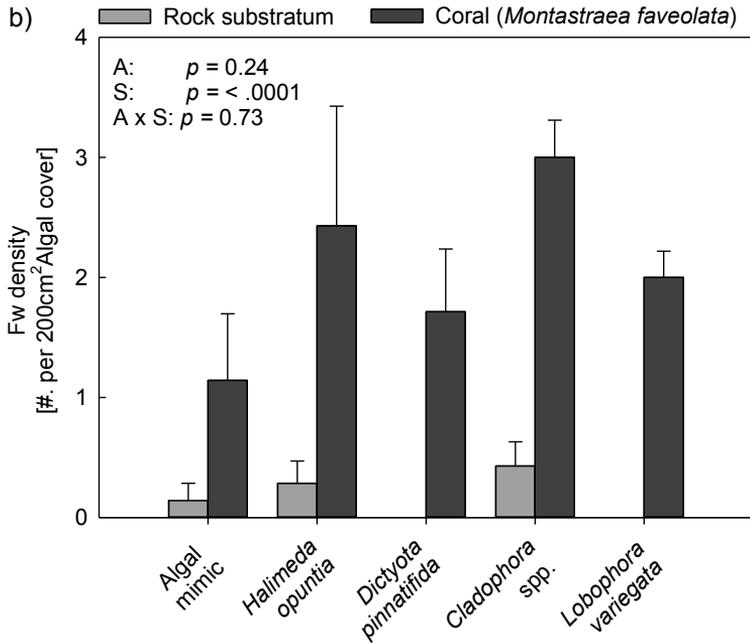


Fig. 1b) *Hermodice carunculata* abundances found in mesh nets filled with different macroalgae or artificial algal mimics (Means \pm SE) and deployed in the drop off area on both rock and coral (*Montastraea faveolata*). All nets were retrieved after 21 d during those experiments (n = 7 replicates, each). Data were analyzed by a Scheirer-Ray-Hare test. Letters refer to different macroalgal treatments (A), substrate types (S), and the interaction of both factors (A x S).

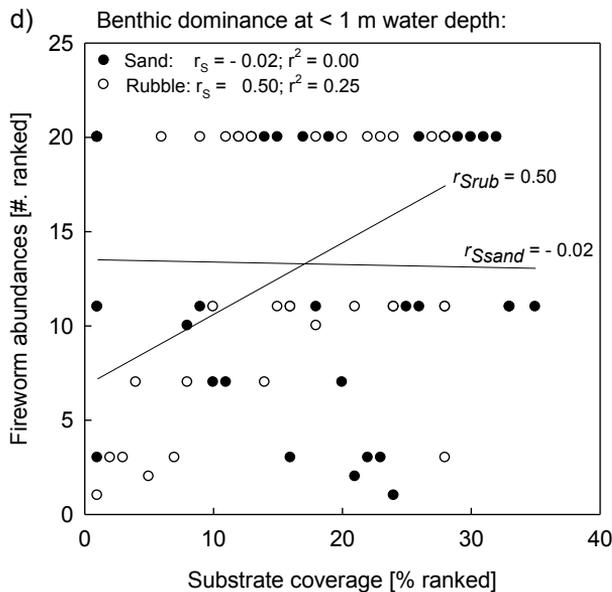
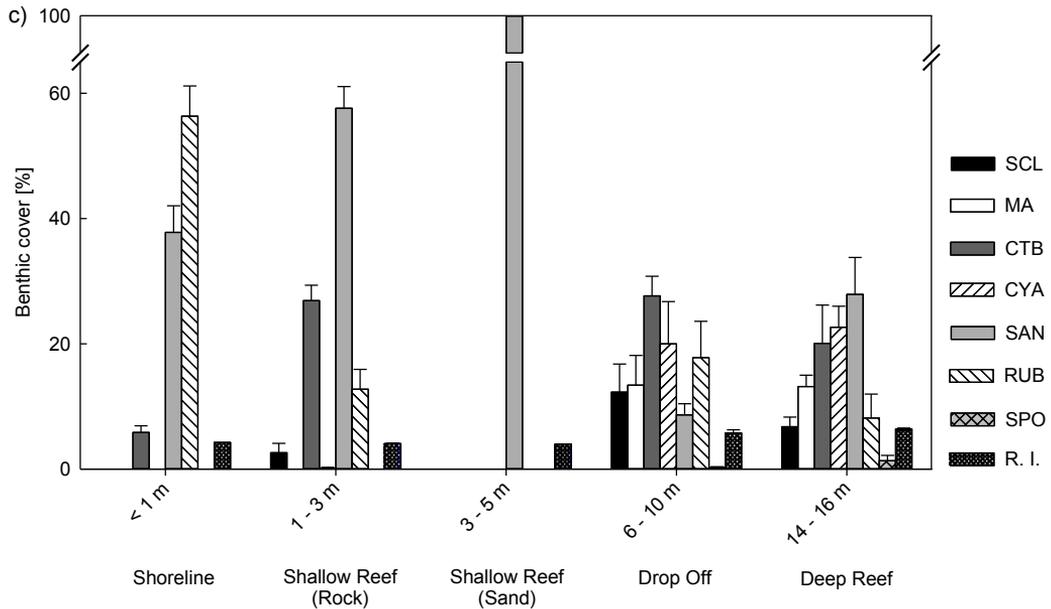


Fig. 1c) Benthic functional group composition (mean percent cover \pm SE) across the depth gradient at the experimental site Buoy Zero. SCL: Scleractinian corals; MA: Macroalgae (> 0.5 cm canopy height); CTB: Crustose algae, turf algae, bare substrate; CYA: Cyanobacterial mats; SAN: Sand; RUB: Rubble; SPO: Sponges; R. I.: Rugosity index. Data derived from 4 m quadrat surveys around each deployed algal mesh net ($n = 7$; number of nets treatment⁻¹).

d) Spearman's Rank analyses assessed correlations for fireworm abundances at the shoreline (< 1 m water depth), and the functional groups Rubble and Sand (%) along the experimental depth gradient. Data was rank transformed prior to analysis.

Nets deployed in the drop off area revealed no significant differences in fireworm densities between the different macroalgae or the algal mimic offered as microhabitat, regardless of rock or coral substratum as underlying substratum ($0 - 0.3 \pm 0.2$ and $1.1 \pm 0.6 - 3 \pm 0.3$ individuals per 200 cm^2 algal cover; respectively). However, there was a strong increase in fireworm numbers when coral colonies were used as underlying substrates (Scheirer-Ray-Hare test, $P < 0.001$ for substratum, $P = 0.24$ for algal species, $P = 0.73$ for interaction) (Fig. 1b).

The benthic community structure at the shoreline ($< 1 \text{ m}$ water depth) showed sand and rubble to be the dominant functional groups (37.8 ± 4.3 and $56.4 \pm 4.8 \%$, respectively), followed by sand only in the shallow reef areas from $2 - 6 \text{ m}$ water depth ($57.6 \pm 3.5 \%$ to 100% total cover) (Fig 1c). CTB dominated the drop off area ($27.6 \pm 3.1 \%$), whereas sand and cyanobacterial mats dominate deeper reef areas (27.9 ± 5.9 and $22.6 \pm 3.4 \%$, respectively). Coral cover was found to be highest in the drop off area ($12.3 \pm 4.5 \%$). Fireworm abundances were tested for correlation with functional groups for the shallow reef zone ($< 1 \text{ m}$ water depth) only. A positive correlation was found with rubble ($r_s = 0.50$), no correlation was found with sand ($r_s = - 0.02$) (Fig. 1d).

Fireworm foraging efficiency & Food preferences

The maximum *H. carunculata* densities were observed in nets retrieved within 24 and 48 h after deployment (2.7 ± 0.4 and 2.4 ± 0.4 individuals per 200 cm^2 algal cover) (Kruskal-Wallis ANOVA on ranks, $H = 23.7$, $df = 4$, $P = 0.001$; for multiple comparisons see Appendix 1) (Fig 2a). In the subsequent fireworm agglomeration & food choice experiment, nets were therefore retrieved after 48 h.

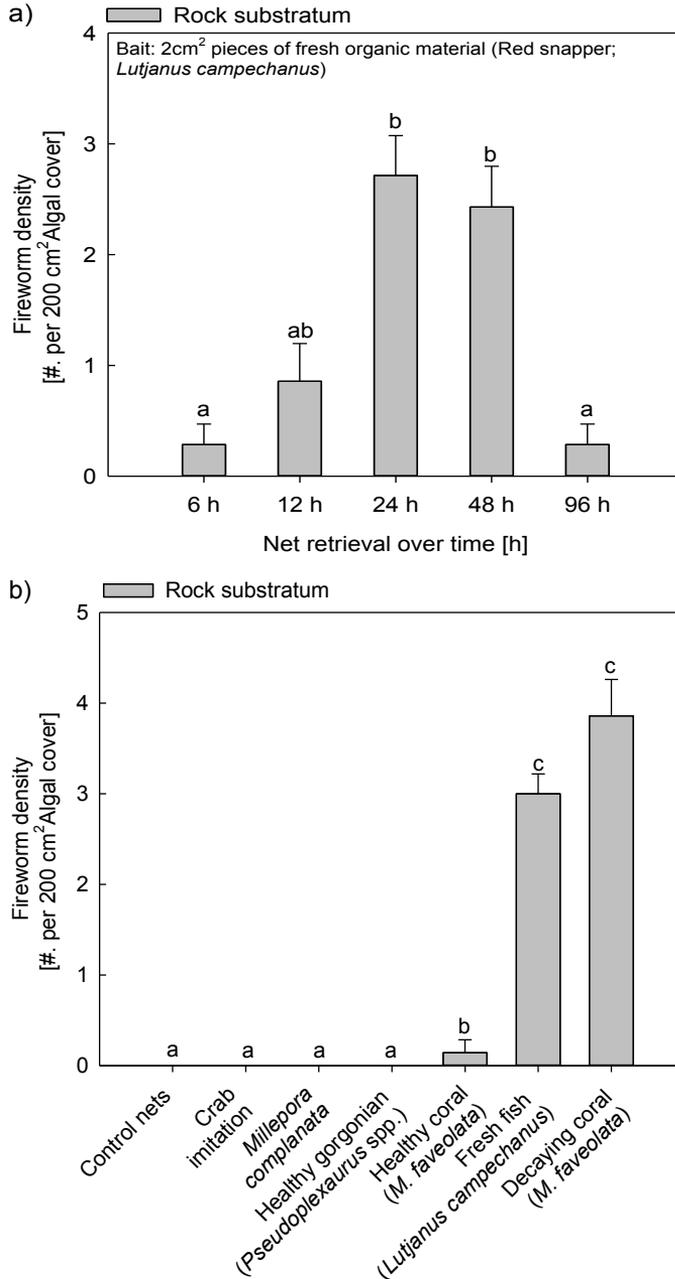


Fig. 2a) Attraction and agglomeration of *Hermodice carunculata* by bait over time. Small pieces (2 cm²) of fresh organic material (Red Snapper, *Lutjanus campechanus*) were enclosed in mesh nets filled with *Halimeda opuntia* and retrieved after 6, 12, 24, 48, and 96 h (n = 7 replicates). **b)** Attraction of *H. carunculata* by different food sources. Small pieces (3

cm²) of different food sources were enclosed in mesh nets filled with *H. opuntia* and retrieved after 48 h (n = 7 replicates). Bars indicate fireworms found per net (Means ± SE). Data on fireworm abundances were analyzed by Kruskal-Wallis ANOVA on Ranks. Letters indicate homogeneous subgroups by multiple comparison z value tests (Fig 2a: a - b; Fig. 2b: a - c). The algae in the control treatment were cleaned of potential food sources before deployment. Coral fragments (*Montastraea faveolata*) were left in small buckets without water exchange for 2 d to allow the fragments to start decaying. **c)** Ex situ photograph: *H. carunculata* feeding on decaying tissue of *M. faveolata*. The buccal mass, a muscular area comprising the pharynx and oesophagus, is fully everted over one polyp, while rippling muscles and secreted enzymes break down the coral tissue and ingest it. Arrows indicate septa depleted of coral tissue due to fireworm induced bite marks. Scale 0.5 cm.



No fireworms were found in the control treatments (no food enclosed), neither in nets with enclosed pieces of gorgonians (*Pseudoplexaura* spp.), hydrozoans (*M. complanata*) or imitation crab meat (Fig. 2b). One individual was found in a net with a healthy coral fragment (0.1 ± 0.1 individuals per 200 cm² algal cover). Nets with fresh organic material (*L. campechanus*), and decaying coral fragments revealed quite large fireworm agglomerations after 48 h (2.0 ± 0.4 and 3.1 ± 0.6 individuals per 200 cm² algal surface area, respectively). The latter two treatments differed statistically from all other treatments (Kruskal-Wallis ANOVA on ranks, $H = 45.1$, $df = 6$, $P < 0.001$; for multiple comparisons see Appendix 1).

Stable isotope and C:N analysis

Isotopic composition (Means ± SE) of fireworm specimens collected during the experiments, i.e. along the depth gradient are displayed as bi-plot ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$)

(Fig. 3a; Means (\pm SE) shown in Table 1). With regard to $\delta^{15}\text{N}$ signatures, the additional group of specimens collected from the channel to Buoy Zero Bay clustered most closely with specimens collected from the shoreline area < 1 m water depth. All specimen groups collected from other reef zones showed similar $\delta^{15}\text{N}$ ranges except for both groups collected at the drop off area (6 – 10 m water depth; Exp. 1a and 1b). For comparison, we added values of specimens collected from another patch reef named Spanish Water and from the channel leading into the adjacent bay (SW and SW Bay, respectively). These specimens clustered closely together with the ones from Buoy Zero Bay. Interestingly, fireworms collected in the reef area (6 – 10 m water depth) of the patch reef at Spanish Water did not cluster with the Buoy Zero congeners, but with the majority of Buoy Zero fireworm groups.

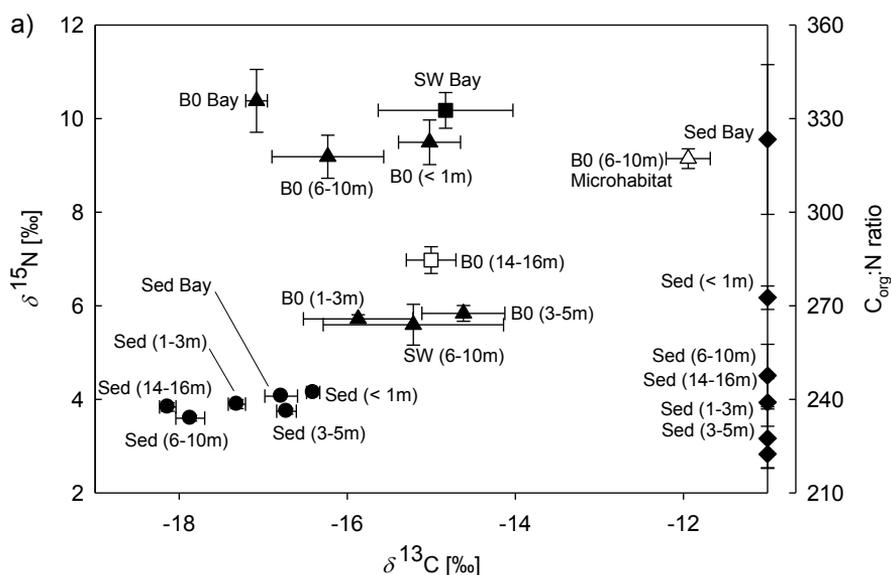


Fig. 3 a) Isotopic bi-plot ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of *Hermodice carunculata* specimens collected during the *in situ* experiments along the depth gradient at the patch reef Buoy Zero and in the adjacent channel to Buoy Zero Bay (B0 and B0 Bay, respectively) (Means \pm SE; $n = 72$ in total, see table 1 for details). Isotope signatures and $C_{\text{org}}:\text{N}$ ratios from sediment samples ($n = 3$ each) and from specimens collected from the drop off area (6 – 10 m) of the patch reef “Spanish Water” and its adjacent bay channel are added to the graph (SW and SW Bay, respectively; $n = 11, 9$).

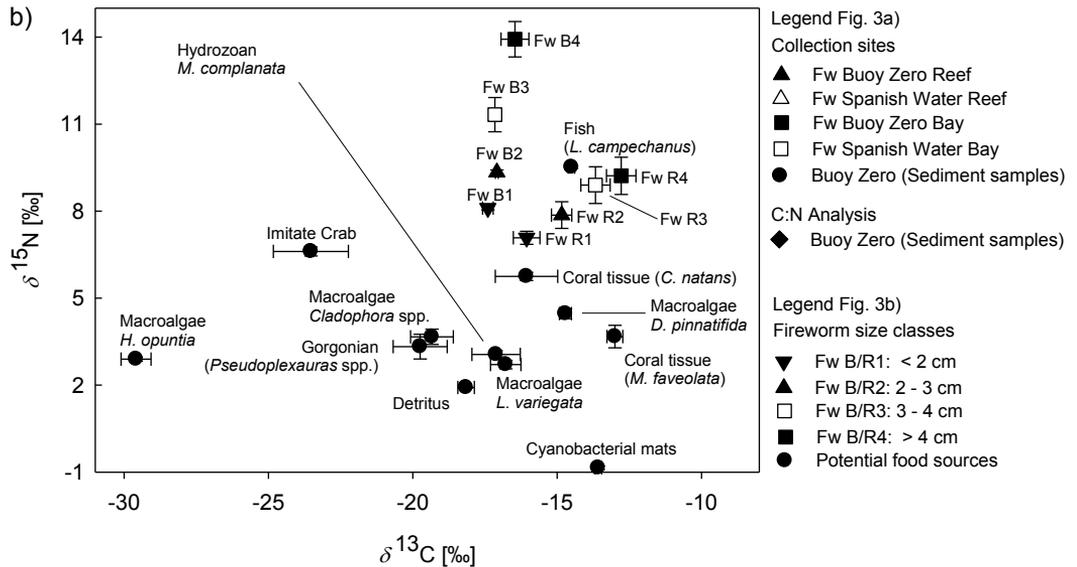


Fig. 3 b) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ stable isotope composition of fireworms (from the patch reef Buoy Zero and the adjacent channel only) (Means \pm SE), after pooling and regrouping in 4 different size classes (Fw “R1 - 4”: see table 1 for replicate numbers). Isotope compositions of potential food sources, collected at Buoy Zero, are added to the graph (Means \pm SE; n = 4 each).

The $\delta^{13}\text{C}$ values of all groups along the water depth gradient and from both reef sites and bay channels were comparable and ranged from -14.6 ± 0.5 to -17.1 ± 0.1 ‰. The group B0 microhabitat (6 – 10 m water depth; Exp. 1b) with Means of -11.9 ± 0.3 ‰ formed the only exception. The stable isotope composition of sediments across the water depth gradient were very similar and ranged from -18.1 ± 0.1 to -16.4 ± 0.1 ‰ and from 3.58 ± 0.1 to 4.1 ± 0.1 ‰ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively.

Furthermore, all fireworms collected during the experiments were pooled and regrouped into four different size classes (Fig. 3b; Means \pm SE shown in table 1). Fireworm groups showed an increase of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values with increasing size class (-16.1 ± 0.5 to -12.8 ± 0.5 and 7.1 ± 0.2 to 9.2 ± 0.6 ‰, respectively).

Table 1. Stable isotope ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) and organic matter content ($\text{C}_{\text{org}}:\text{N}$ ratio) values for fireworm specimens and sediment samples collected from the experimental site Buoy Zero and the adjacent channel to Buoy Zero Bay. Values are displayed according to a) experimental collection site (Fig. 3a) and b) pooled and regrouped to 4 different size classes (Fig. 3b). n = numbers of replicates within each group.

Figure 3a Experimental site / Collection site	$\delta^{13}\text{C}$		$\delta^{15}\text{N}$		CN ratio		n
	Avg	SE	Avg	SE	Avg	SE	
FW- B0 Bay channel	-17.08	0.13	10.38	0.67	4.19	0.16	11
FW- Shoreline (< 1m)	-15.02	0.37	9.49	0.48	4.22	0.45	7
FW- Shallow Reef Rock (1 - 3 m)	-15.87	0.65	5.72	0.09	3.80	0.17	6
FW- Shallow Reef Sand (3 – 5 m)	-14.62	0.49	5.84	0.17	4.45	0.14	6
FW- Drop Off (6 – 10 m)	-16.23	0.66	9.18	0.46	5.34	0.35	15
FW- Deep Reef (14 – 16 m)	-15.00	0.30	6.97	0.29	5.07	0.29	17
FW- B0 Microhabitat	-11.94	0.26	9.14	0.21	4.36	0.25	9
FW-SW Bay channel	-14.83	0.80	10.18	0.38	3.88	0.10	9
FW-SW DO (6 – 10 m)	-15.21	1.07	5.59	0.44	3.88	0.15	11
B0-Sediment B0 Bay channel	-16.79	0.19	4.07	0.01	323.29	24.01	3
B0-Sediment B0 Shoreline (< 1 m)	-16.40	0.08	4.14	0.04	272.57	3.75	3
B0-Sediment SR Rock (1 - 3 m)	-16.72	0.12	3.74	0.06	222.46	4.59	4
B0-Sediment SR Sand (3 - 5 m)	-17.31	0.10	3.90	0.09	227.48	9.41	3
B0-Sediment DO (6 - 10 m)	-17.87	0.17	3.60	0.06	247.60	10.02	3
B0-Sediment DR (14 - 16 m)	-18.14	0.10	3.84	0.09	238.98	7.62	3

Figure 3b Fireworm / Potential food sources	$\delta^{13}\text{C}$		$\delta^{15}\text{N}$		CN ratio		n
	Avg	SE	Avg	SE	Avg	SE	
Fw size class 1: B0 < 2 cm	-16.06	0.46	7.08	0.22	4.77	0.25	22
Fw size class 2: B0 2 -3 cm	-14.84	0.35	7.86	0.46	4.66	0.20	23
Fw size class 3: B0 3- 4 cm	-13.67	0.51	8.90	0.63	4.92	0.20	9
Fw size class 4: B0 > 4 cm	-12.78	0.51	9.22	0.64	4.87	0.70	7
Fw size class 1: B0 Bay < 2 cm	-17.40	0.18	8.10	0.06	4.69	0.32	3
Fw size class 2: B0 Bay 2 -3 cm	-17.09	0.05	9.34	0.08	4.15	0.24	3
Fw size class 3: B0 Bay 3- 4 cm	-17.16	0.09	11.33	0.59	3.99	0.10	3
Fw size class 4: B0 Bay > 4 cm	-16.46	0.48	13.93	0.61	3.81	0.16	2
MA - <i>Halimeda opuntia</i>	-29.59	0.52	2.90	0.07	13.14	0.63	4
MA - <i>Dictyota pinnatifida</i>	-14.72	0.21	4.46	0.17	15.39	0.90	4
MA - <i>Lobophora variegata</i>	-16.78	0.53	2.71	0.14	36.27	4.74	4
MA - <i>Cladophora sp.</i>	-19.34	0.74	3.67	0.26	12.01	0.77	4
Fish (<i>Lutjanus campechanus</i>)	-14.50	0.11	9.52	0.04	3.29	0.16	4
Imitation Crab meat	-23.53	1.30	6.61	0.15	19.67	0.79	4
Gorgonian (<i>Pseudoplexaura sp.</i>)	-19.75	0.94	3.33	0.43	5.66	0.51	4
Coral (<i>Colpophyllia natans</i>)	-16.06	1.08	5.75	0.14	6.31	1.74	5
Coral (<i>Montastraea faveolata</i>)	-13.00	0.27	3.67	0.39	5.33	0.83	4
Cyanobacterial mat	-13.58	0.12	-0.85	0.06	6.77	0.76	4
Hydrozoan (<i>Millepora complanata</i>)	-17.12	0.84	3.06	0.13	10.66	3.99	5
Detritus	-18.15	0.29	1.92	0.02	13.08	1.99	4

Specimens collected in the inland channel of Buoy Zero Bay show similar pattern, yet higher mean values, ranging from -17.4 ± 0.2 to -16.5 ± 0.5 and from 8.1 ± 0.1 to 13.9 ± 0.6 ‰, for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures, respectively. The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic signatures derived from a variety of potential food sources were comparable to those known from literature.

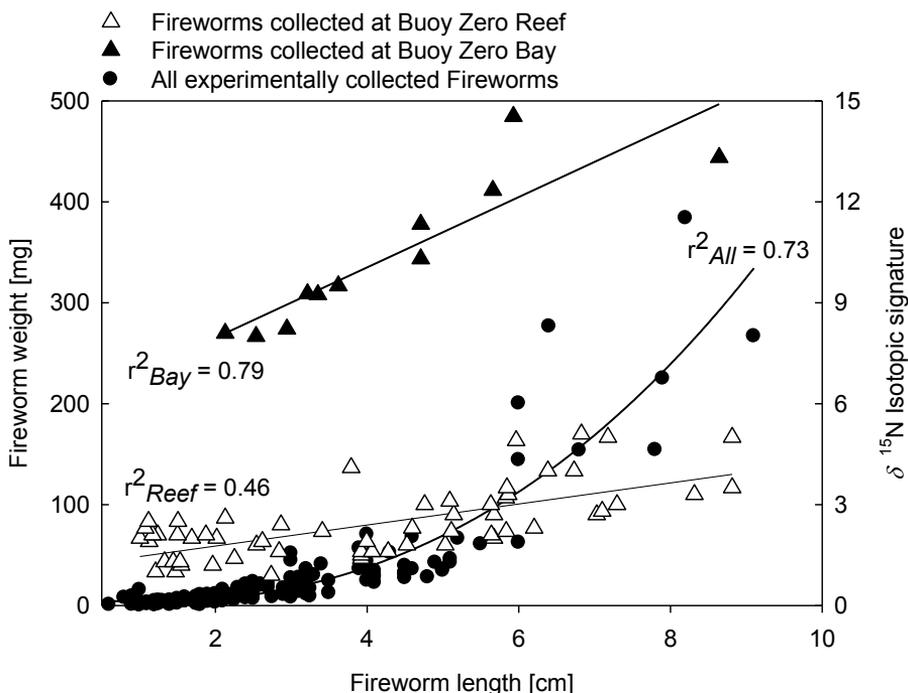


Fig. 3 c) Length-weight relationship of *Hermodice carunculata* individuals collected at Buoy Zero during different experiments in 2010/11 ($n = 191$). Superimposed, Pearson's product correlation analyses for Fw $\delta^{15}\text{N}$ values vs. body length from Buoy Zero reef and Buoy Zero bay individuals ($n = 61$ and 11 , respectively).

The length-weight relationship of fireworm individuals collected during various experiments in 2010 and 2011 increased exponentially. Fireworm body size was positively correlated with the $\delta^{15}\text{N}$ isotopic signature of individuals collected from Buoy Zero Reef and Bay areas (Pearson product-moment correlation $r_{pReef} = 0.68$ and $r_{pBay} = 0.89$, respectively) (Fig. 3c).

Discussion

Distribution pattern of Hermodice carunculata

The shallowest reef zone of the patch reef Buoy Zero, i.e. the shoreline (< 1 m water depth), displayed very low habitat complexity (56 and 38 % rubble and sand coverage; respectively), yet highest fireworm abundances in the MADs. Organic matter and stable isotope composition of shoreline samples indicated highly refractory surface sediments ($C_{org}:N = 272 \pm 4$; $\delta^{15}N$ range: 3.58 ± 0.1 to 4.1 ± 0.1 ‰), although isotope analysis of fireworms showed remarkable high $\delta^{15}N$ signatures of 9.5 ± 0.5 ‰. This may be explained by the role of biogenic-derived carbonate sediments which play a crucial role for element cycling within reef ecosystems (Rasheed et al. 2002; Wild et al. 2004a). High water perturbation at the shoreline, the permeability of coarse reef sands, and sediment-associated microbial communities enhance rapid re-mineralization processes across the sediment (Schoettner et al. 2011; Wild et al. 2004b).

Bottom-dwelling invertebrates thus may be attracted into the sediment, and Werner et al. (2006) suggested that mineralization rates may even be controlled by benthic fauna present within the sediment (see also Lindsay 2009). We assume the elevated isotopic signatures of fireworms to derive from feeding pattern on the diverse meiofauna and / or degraded organic material in deeper sediment regions. Observations of fireworms emerging from sediments (A. Wolf, pers. observation), and Jumars (1993) who described sediment-associated dietary patterns as common in bottom dwelling polychaetes lend further support to this hypothesis. The paucity of potential predators likely facilitates large fireworm agglomerations in these reef zones, as discussed below.

Microhabitat specificity

Macroalgae are known to host a variety of marine invertebrates (García-Ríos et al. 2008; Naim 1988). With regard to microhabitat specificity, our results indicate that all tested macroalgae and the algal mimic can serve as potential refuge for *H.*

carunculata, but this depends on the direct surrounding of the macroalgae. The much higher fireworm abundances in algal clumps deployed on coral substratum suggest a strong preference for refuges in close proximity to potential food sources. Importantly, most underlying coral colonies showed signs of tissue deterioration after 21 d in contact with macroalgae (data not shown, also see Wolf et al. 2012). Fireworms likely were attracted by decaying coral tissue as potential food source, confirming observations of experiment 2 b and earlier work of the authors (Wolf and Nugues, *in review*).

The differences in fireworm numbers between the two substrate types raise the question as to whether results from experiment 1a represent accurate fireworm abundances for the drop off area of a patch reef. If so, densities are very low (0.3 individuals per 200 cm² algal cover), and the agglomerations in experiment 1b are overestimates rather than actual densities caused by the fireworms' ability to detect preferred food sources such as decaying coral tissue even from a wide distance. Otherwise, results in exp. 1a are true underestimates of actual densities, more accurately represented by experiment 1b; and fireworms, although actually present within that area, prefer even more cryptic habitat niches such as crevices and only seek shelter in *H. opuntia* clumps in the presence of a preferred food source. Furthermore, many macroalgae including the ones tested in our experiment are known to exert allelochemical compounds (Paul and Fenical 1986; Rasher and Hay 2011; Smith et al. 2006). The effects of algal-derived deterrents on marine invertebrates are not well-studied, but it is possible that epifauna even avoids these refuges or only uses them as temporary shelter.

Foraging efficiency & Feeding preferences

Chemoreception, which includes both olfaction and gustation, mediates ecologically important behavior such as defense, reproduction, and feeding in many marine organisms, including polychaetes (Fauchald and Jumars 1979; Hay 2009). Undirected *in situ* foraging behavior of *H. carunculata* was recorded for sea anemones (Lizama and Blanquet 1975) and hydrozoans (Witman 1988), and

Lindsay (2009) emphasized the stronger importance of gustation over olfaction during the foraging processes of amphinomids. Nevertheless, experiment 1b, 2a and 2b prove *H. carunculata* to possess excellent olfactory organs and to be able to detect food sources within a relatively short period of time.

Experiment 2b revealed strong preferences for fresh and decaying organic material, underlining its role as omnivorous scavenger. We cannot exclude the possibility that fresh organic material and decaying coral tissue exerted higher concentrations of attracting olfactory compounds, compared to hydrozoans and gorgonians. Healthy scleractinian corals exhibit a variety of defense mechanisms such as chemical deterrents and nematocysts (Gochfeld 2004; Gore 1984), which may explain the absence of fireworms in the respective algal clumps. Still, these mechanisms do not exclude scleractinian corals (Ott and Lewis 1972) or hydrozoans (Lewis and Crooks 1996) from predation by *H. carunculata*. The observed preferences may thus be explained by a release of olfactory attractants, a reduction in feeding deterrents (e.g. nematocysts) and/or higher energy rewards in damaged coral tissue (McIlwain and Jones 1997).

Dietary pattern

Most individuals revealed consistent pattern with regard to their trophic level based on isotopic signatures, a measure commonly used to describe dietary pattern in marine organisms (Fry 2008). Assuming a change of 3 - 4 ‰ in $\delta^{15}\text{N}$ isotope value to reflect the transition from one trophic level to the next, the diet of fireworms would generally comprise detritus, turf- and macroalgae, but also gorgonians and hydrozoans, which confirms earlier and comparable studies (Jumars 1977; Witman 1988; Vreeland and Lasker 1989; Ott and Lewis 1972). Lewis (2009) found chloroplast aggregations during gut analysis, confirming our results. The relatively higher $\delta^{15}\text{N}$ values for shoreline, Buoy Zero - and Spanish Water Bay individuals suggest a diet which additionally comprises sediment-derived organic matter and / or biodegradable and decaying organic material.

The differences in $\delta^{15}\text{N}$ isotopic signatures between fireworms collected from the drop off areas of Buoy Zero and Spanish Water may be attributed to the apparent differences in health condition of certain coral species. The reef building corals *M. faveolata*, *Diploria strigosa* and *Colpophyllia natans* exhibited considerable amounts of white plague like signs at Buoy Zero, but not at Spanish Water, which is commonly considered pristine for Caribbean standards (A. Wolf, pers. observation). Our data show that especially decaying coral tissue constitutes a highly preferred food source for *H. carunculata*. If these deteriorating conditions persisted over extended periods of time, fireworms in the drop off area of Buoy Zero may have oriented their diet more towards scleractinian corals, which may be reflected by higher isotopic signatures.

When grouped into four different size classes, the increase in $\delta^{15}\text{N}$ isotopic signature (7.0 - 10.5 ‰) from smallest to largest individuals represents one trophic level within the food web, which suggests an ontogenetic shift in diet. These findings are independent of the respectively inhabited reef zone and also hold for specimens collected in the adjacent channels depauperate of corals. Ontogenetic food shifts are common for invertebrate corallivores, as recorded for the muricid gastropods *Nucella emarginata* (Gosselin 1997) and *Drupella cornus* (Schoepf et al. 2010), and the grapsid crab *Pachygrapsus crassipes* (Sousa 1993). Likewise, juvenile *N. emarginata* revealed preferences for smaller, but not energetically more preferable prey, and differences were attributed to habitat constraints rather than to nutritional preference (Gosselin and Chia 1996). Resource utilization ability and predation risk are generally related to body size, and many species undergo extensive ontogenetic shifts in habitat use (Werner and Gilliam 1984). Small-sized organisms thus decrease predation risk by increasing the use of refuges (Eklov and Diehl 1994; Mittelbach 1981), but concomitantly narrowing down their potential food repertoire. Small fireworms are extremely cryptic organisms which often forage within defined microhabitats for potential food sources, but also to minimize their predation risk. A similar use of microhabitats was found for juvenile

individuals of *N. emarginata* (Gosselin 1997), *D. cornus* (Schoepf et al. 2010) and *Drupella fragrum* (Cumming 1999).

There are a few predators known to prey on *H. carunculata*. The predatory anemone *Phyllactis flosculifera*, abundant in sand beds, was observed to feed on *H. carunculata* (D. Meyer, pers. comm. in Sebens 1982). We observed bluehead wrasses (*Thalassoma bifasciatum*) and yellowhead wrasses (*Halichoeres garnoti*) to prey on *H. carunculata* (A. Wolf, pers. observation). Predation was highly dependent on fireworm size, and specimens above a certain size (> 4 cm) were not preyed upon anymore. *H. carunculata* exhibit an exponential increase in body weight once they exceed a certain body length. Fireworms likely actively increase their foraging return by widening their habitat and simultaneously extending their food repertoire as soon as the few existing predators do not pose an immediate predation risk anymore, confirming our findings of highest isotopic signatures for largest individuals.

Ecological relevance

Most studies on *H. carunculata* and its impact on scleractinian corals were relatively dated (Marsden 1962, 1963a, 1963b; Ott and Lewis 1972). Impeded recovery of *Acropora* spp. and vertical growth in fringing reefs were attributed to *H. carunculata* (Knowlton et al. 1990; Witman 1988). Nevertheless, Ott and Lewis (1972) considered the impact of fireworms on coral community structure as theoretically significant, but negligible due to their limited abundance.

Our results provide experimental evidence for a strong variability in distribution patterns of *H. carunculata*. The fireworm prefers the shallowest of reef zones, speculatively due to favorable food accessibility, and hence should be considered as omnivorous detritivores rather than facultative corallivores. Stable isotope data suggest its diet to be oriented towards detritus, turf- and potentially macroalgae. However, although we assume healthy corals to only marginally contribute to their diet, degrading corals appear to be a preferred food source for *H. carunculata*. Studies should continue to shed light on *H. carunculata*'s role as

predator and as prey within the reef system. More broadly, further research on population dynamics of corallivorous invertebrates may prove useful to identify changes in community structure and to better manage remediation measures to maintain reef resilience.

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Author contributions

ATW, MMN and CW designed the study; ATW performed the study, analyzed the data and wrote the manuscript. All authors edited the manuscript and approved the submission.

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Appendix 1:

Table 1 All pair wise multiple comparison Z value tests between treatments for experiments 1a, 2a, and 2b

Exp. 1a Abundance & Habitat	< 1 m	1 - 3 m	3 - 5 m	6 - 10 m	14 - 16 m		
Shallow Reef Shoreline (< 1 m)	-						
Shallow Reef Rock (1 - 3 m)	2.88*	-					
Shallow Reef Sand (3 - 5 m)	2.92*	0.04	-				
Drop Off (6 - 10 m)	3.52*	0.64	0.60	-			
Deep Reef (14 - 16 m)	2.87*	0.01	0.05	0.65	-		
Exp. 2a Foraging efficiency	6 h	12 h	24 h	48 h	96 h		
6 h	-						
12 h	0.94	-					
24 h	3.39*	2.45	-				
48 h	3.10*	2.16	0.29	-			
96 h	0.00	0.94	3.39*	3.10*	-		
Exp. 2b Food preferences	Control	Crab imit.	Hydroz.	Gorg.	Healthy Coral	Fish	Decayed Coral
Control (no food source)	-						
Crab imitation meat	0.00	-					
Hydrozoan (<i>M. complanata</i>)	0.00	0.00	-				
Gorgonian Pseudoplexaura sp.	0.00	0.00	0.00	-			
Healthy Coral (<i>M. faveolata</i>)	0.33	0.33	0.33	0.33	-		
Fish (<i>L. champechanus</i>)	3.04*	3.04*	3.04*	3.04*	2.71	-	
Decayed coral (<i>M. faveolata</i>)	3.51*	3.51*	3.51*	3.51*	3.18*	0.47	-

* significance level: $P = 0.05$

2

The curious case of *Hermodice carunculata*: an amphinomid polychaete exhibiting high population connectivity throughout the Atlantic Ocean

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Abstract

Over the last few decades, molecular studies of polychaete annelids have revealed deep genetic divergences between morphologically indistinguishable populations that were once considered the same species, a phenomenon known as cryptic speciation. Molecular work on fireworms (family: Amphinomidae) has revealed the presence of cryptic species complexes in both the Atlantic and Pacific Oceans. Recently, morphological analysis indicated that the bearded fireworm, *Hermodice carunculata* Kinberg 1857, is not truly a single species, and the species name *Hermodice nigrolineata* Baird 1870 has been reinstated to contain the Eastern Atlantic populations. Here, we present a molecular analysis of mitochondrial cytochrome *c* oxidase subunit I (COI) and 16SrDNA sequence data as well as the nuclear internal transcribed spacer (ITS) for *Hermodice* throughout its oceanographic range in the Atlantic Ocean, including the Mediterranean Sea and the Gulf of Mexico. Phylogenetic analyses of mitochondrial loci revealed a private lineage found only in the Mediterranean Sea, but no barriers between Eastern and Western Atlantic populations were indicated. Additionally, morphological differences between the specimens used in our study were not reliable and did not correlate well with the genetic data. Our results do not support the current distinction between Eastern and Western Atlantic *Hermodice* populations and suggest that, unlike other amphinomids, *H. carunculata* is a cohesive species with high population connectivity across the Atlantic Ocean.

Introduction

Historically, it has been assumed that marine species generally exhibit a higher degree of connectivity among geographically distant populations than terrestrial species (Sanford and Kelly, 2011). The long-lived larval stages of most marine fauna coupled with an apparent absence of barriers to dispersal were thought to allow for sufficient gene flow to prevent populations from diverging (Grosberg and Cunningham, 2001). Subsequent research, however, has indicated that planktonic dispersal and a paucity of oceanographic barriers do not always lead to highly-connected populations (Levin, 2006; Palumbi, 2004; Sanford and Kelly, 2011).

In the last few decades, molecular genetic studies on numerous marine taxa have been used to distinguish cosmopolitan species, which have a very large geographic range, from complexes of cryptic species, consisting of multiple morphologically indistinguishable but genetically distinct lineages. For example, an early electrophoresis study revealed distinct alleles in local populations of *Capitella capitata*, implying the existence of a cryptic species complex along the Atlantic coast (Grassle and Grassle, 1976). As direct DNA sequencing became more widespread, molecular work on polychaete annelids, mainly based on Cytochrome *c* oxidase subunit I (COI) sequence data, has also revealed deep divergences (5-15%) among congeners that were formerly considered the same species (Barroso et al., 2010; Borda et al., in review; Pires et al., 2010; Schulze, 2006). Additionally, morphological work which revealed differences between lineages purported to be conspecific has led to taxonomic revisions (Pires et al., 2010; Yáñez-Rivera and Salazar-Vallejo, 2011).

Fireworms (Family: Amphinomidae) are a lineage of jawless polychaetes with diverse lifestyles and, in some cases, striking coloration. Many species are armed with calcareous, harpoon-like setae capable of piercing the soft tissue of predators and causing a painful burning sensation. Amphinomids are also reported to have a larval stage known as a rostraria (Bhaud, 1972; Mileikovsky, 1961). This

larval form is poorly studied but is presumed to be planktotrophic and teleplanic, making it a possible medium for large-scale dispersal, gene flow and high population connectivity. Nonetheless, analysis of COI sequence data has shown that the fireworm *Eurythoe complanata*, whose range extends into the Atlantic and Pacific oceans, is actually a complex of at least three very genetically distant lineages, despite the absence of obvious morphological differences among them (Barroso et al., 2010). Similarly, the genus *Archinome*, associated with hydrothermal vents worldwide, consists of six genetically distinct lineages for which no morphological distinctions have been identified (Borda et al., in review).

Fireworms in the genus *Hermodice*, commonly known as bearded fireworms, are facultative corallivores (Lewis, 2009) found in reef habitats throughout the Atlantic Ocean, including the Gulf of Mexico, the Caribbean Sea, the South American and African Atlantic coasts, and the Mediterranean and Red Seas. Their destructive feeding behavior is known to stunt the growth of the hydrocoral *Millepora complanata* in the Caribbean Sea (Whitman, 1988). Additionally, in the Mediterranean Sea, *Hermodice* acts as a winter reservoir and possibly a vector for the coral-bleaching pathogen *Vibrio shiloi* (Sussman et al., 2003). Initially, the genus contained eight species, including *H. striata* Kinberg, 1857 and *H. carunculata* (Kinberg, 1857), previously *Aphrodita carunculata* (Pallas, 1766). Over time, former members of the genus were either reclassified as junior synonyms of *H. carunculata* or moved to the genus *Pherecardia* based on caruncle morphology and *Hermodice* became a monotypic genus. However, Yáñez-Rivera and Salazar-Vallejo (2011) found that Eastern and Western Atlantic populations exhibit differences in the abundance of branchial filaments as well as the shape of the anal lobe and, based on these characters, they reinstated the name *H. nigrolineata* (Baird, 1870) to contain all *Hermodice* from the Eastern Atlantic, as well as populations in the Mediterranean Sea and the Gulf of Guinea. Based on the vast oceanographic range for this genus, they suspected that more cryptic species within Eastern and Western Atlantic populations might be revealed by molecular analyses (Yáñez-Rivera and Salazar-Vallejo, 2011).

To determine whether there is molecular support for the distinction between the two morphospecies proposed by Yáñez-Rivera and Salazar-Vallejo (2011), and to test whether more cryptic species exist in the Greater Caribbean or East Atlantic, we conducted a phylogeographic analysis of COI and 16S ribosomal DNA (16S) sequence data. To assess the level of genetic connectivity among populations, we calculated their average genetic distances as well as genetic diversity statistics based on an analysis of molecular variance (AMOVA). In addition, to test whether genetic distances correlate with morphology, we used our own samples to evaluate differences in the number of branchial filaments, an important feature used to distinguish *H. carunculata* and *H. nigrolineata* as described by Yáñez-Rivera and Salazar-Vallejo (2011). We hypothesize that branchial filament abundance is a function of dissolved oxygen concentration, and we present GIS data to illustrate this possible link.

Material and Methods

Specimen Collection/ Storage

SCUBA divers hand-collected live *Hermodice* specimens from temperate coral reefs in several locales across the Atlantic Ocean (Table 1). In the East Atlantic, *Hermodice* were taken from several locations in the northern Gulf of Mexico and Caribbean Sea as well as Rocas Atoll, Rio de Janeiro and St. Peter and St. Paul Archipelago. Specimens in the alleged range of *H. nigrolineata* were collected from the Mediterranean Sea and from Sao Tomé in the Gulf of Guinea. Many of the specimens to be used for sequence analysis were preserved in ethanol immediately after collection, and some were frozen while being transported to a lab. Frozen specimens had deteriorated and were unusable for morphological analysis. All specimens as well as DNA vouchers have been deposited in the Los Angeles County Natural History Museum.

Table 1 A list of locations sampled during this study as well as the number of specimens used in each analysis

Locale	Coordinates	Tree ID	Arlequin (COI)	Arlequin (16s)	Phylogeny	Genetic Distance (COI)	Genetic Distance (ITS)	Morphological Analysis
Bahamas	24.32°N 76.28°W	BA	3	-	3			
Belize	17.48°N 88.52°W	BE			1			
Bocas del Toro	9.33°N 82.25°W	BDT	16	18	16	16		5
Crete	35.21°N 24.91°E	CR	15	11	9	15	5	13
Curaçao	12.18°N 69.00°W	CU	8	10	8	8		
Florida Keys	24.67°N 76.28°W	FK		-	2			
Flower G. Banks	27.83°N 93.83°W	FGB	9	11	9	9	2	4
Malta	35.90°N 14.46°E	MA	6	7	7	6		6
Panama City, FL	30.17°N 85.66°W	PC	5	10	5	5		12
Quintana Roo, Mex.	19.60°N 87.91°W	QR		-	2			
Rio de Janeiro	22.91°S 43.24°W	RDJ	3	3	3			2
Rocas Atoll	3.87°S 33.82°W	RA	15	19	15	15		5
St. Thomas, U.S.	18.43°N 64.65°W	VI	9	10	8	9		2
Virgin Islands								
St. Peter/ St. Paul Archipelago	0.92°N 29.34°W	SPSPA	17	18	17	17		5
Sao Tomé	0.32°N 6.67°E	ST	5	6	5	5		1
Sonnier Bank	28.30°N 92.45°W	SB	8	9	8	8		

DNA Purification and Sequencing

A small tissue sample was removed from either the dorsal or ventral side of a large segment of each worm. For specimens where amplification was initially unsuccessful via dorsal tissue samples, a second sample was taken from ventral tissue, which contains more mitochondrial DNA owing to a higher density of muscle fibers. DNA purification was accomplished using a DNeasy Blood and Tissue Kit (Qiagen) following the protocols provided by the manufacturer.

All sequences from Bocas del Toro, Brazil, Saint Peter and Saint Paul Archipelago, and Sao Tomé were generated at the Federal University of Rio de Janeiro. Sequences from Curaçao were generated at the Leibniz Center for Tropical Marine Ecology in Bremen, Germany. All other sequences were generated at Texas A&M University at Galveston. For each specimen, fragments of the mitochondrial cytochrome *c* oxidase subunit I (COI) and 16S RNA genes were amplified via PCR. The primers LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3'), HCO 2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer et al., 1994) and COID (5'-TCTGGGTGTCCRAARAAYCARAA-3') (Kojima et al., 1997) were used for COI amplification. For 16S RNA, the primers 16Sa (5'-CGCCTGTTTATCAAAAACAT-3') and 16Sb (5'-CTCCGGTTTGAAGTCAATCA-3') were used (Xiong and Kocher, 1991). For a small subset of the specimens examined, the nuclear Internal Transcribed Spacer (ITS1) was amplified using the primers ITS18SFPOLY (GAGGAAGTAAAAGTCGTAACA), and ITS5.8SRPOLY (GTTCAATGTG TCCTGCAATTC) (Pleijel et al., 2008). PCR reactions were carried out following standard protocols in 25µL volumes with an annealing temperature of 45°C. Cycle sequencing using the BigDye Terminator (Life Technologies) chemistry was conducted in 10 µl volumes, following standard protocols.

Sequences were analyzed using an ABI 3130 Genetic Analyzer, and resulting electro-chromatograms were edited in SequencherTM 4.2 by assembling the forward and reverse fragments from each specimen and deleting the primer regions. All sequences were submitted to GenBank.

Sequence Analysis

Edited sequences were aligned in MEGA 5 (Tamura et al., 2011) using the ClustalW algorithm. The number of distinct haplotypes for each gene and for the combined COI and 16S alignment were calculated using FaBox (Villesen, 2007). Phylogenetic analyses for COI and 16S rDNA were performed using Bayesian Inference (BI), Maximum Likelihood (ML), and Neighbor Joining (NJ). Bayesian Inference was conducted via MrBayes 3.1.2 (Huelsenbeck et al., 2002) using the generalized time-reversible model GTR+G+I, which was selected based on results from jModelTest (Guindon and Gascuel, 2003; Posada, 2008). Maximum Likelihood trees were constructed in raxmlGUI (Silvestro, 2011) using GTR+G as recommended by a previous author (Stamatakis, 2006). For Maximum Likelihood, a thorough bootstrap (10 runs, 1000 pseudo-replicates) was conducted to produce branch support values. Additionally, the COI portion of the concatenated sequence data was partitioned by codon position (first, second, and third) for both Bayesian Inference and Maximum Likelihood analyses. Neighbor Joining was carried out in MEGA using the bootstrap method ($n = 1000$) to calculate branch support. The combined COI and 16S rDNA tree was rooted using specimens of *Eurythoe complanata*, *Cryptonome conclava*, *Hipponoa gaudichaudi*, *Paramphinome jeffreysii*, *Pareurythoe borealis* and *Amphinome rostrata* as outgroups.

Corrected mean genetic distances among and within sample locales were calculated for COI and ITS using Kimura's 2-parameter model (Kimura, 1980). Standard errors for these distances were estimated using 1000 bootstrap replicates. Analyses of molecular variance (AMOVA) were conducted using Arlequin 3.5.1.2 (Excoffier and Lischer, 2010) for COI and 16S rDNA individually. For each gene, two separate analyses were run. One analysis grouped sample locales according to the ranges of the two recently described morphospecies, i.e. Eastern vs. Western Atlantic (Yáñez-Rivera and Salazar-Vallejo, 2011). The other assigned Mediterranean locales to one group and all other locales to the second group. In all cases, a basic AMOVA was computed using 1000 permutations and Kimura's two-parameter model (Kimura, 1980).

Morphological Analysis

A total of 53 specimens from the East (n = 20) and West (n = 33) Atlantic Ocean were examined to determine whether our samples exhibited the morphological distinction in branchial filaments observed by Yáñez-Rivera and Salazar-Vallejo (2011) (Refer to Table 1 for a list of locales examined). Individuals were selected based on their overall condition; specimens that had been frozen prior to ethanol preservation were not used as they were too deteriorated to be scored correctly. Specimens were separated into groups for statistical analysis according to the locales in which they were collected (East vs. West Atlantic). For each individual, the total number of chaetigers was recorded as well as the number of dorsal branchial filaments on the 10th chaetiger posterior to the prostomium for statistical analysis.

Because the total number of chaetigers for certain specimens was insufficient for proper scoring according to previously established criteria (>50 Chaetigers), dorsal branchial filaments on 5 randomly selected but adjacent medial segments were counted for each specimen and then averaged. Ratios of the number of branchial filaments at the 10th chaetiger to the total number of chaetigers were calculated for members of each group. Additionally, the ratio of the average number of medial filaments to the total number of chaetigers was computed and used for similar statistical analyses. Statistics were computed using JMP 9 Statistical Software (SAS, 2007). Mean filament abundances as well as means of the ratios described above were compared for the two groups using the nonparametric Wilcoxon Rank Sums test based on Q-Q plots and Brown-Forsythe statistics for each dataset. A power regression was also computed to determine whether there is a statistical interaction between dorsal filament abundance and the locations where our specimens were collected. Since the specimens were preserved in ethanol, anal lobe morphology could not be used as a reliable criterion for delimiting species and was not examined. A logistic regression was used to compute the probability that they belonged to the Eastern Atlantic group despite their genetic dissimilarity.

Results

Phylogeny

Sequencing analysis resulted in a concatenated alignment of 1122 bp for 119 specimens. Of the 607 COI sites analyzed, 114 were variable and 58 were parsimony-informative. The remaining 515 bp of 16S rDNA sequence data contained 59 variable sites, 12 of which were parsimony informative. The total alignment consisted of 114 haplotypes, of which 111 were singletons (i.e. occurred only in a single individual). Further analysis revealed 103 haplotypes (96 singletons) for COI and 88 haplotypes (77 singletons) for 16S.

Phylogenetic analysis via Bayesian Inference (BI), Neighbor-Joining (NJ), and Maximum Likelihood (ML) resulted in a tree containing two main clades, the larger of them containing multiple subclades that were congruent between all analysis methods (Figure 2). Branch support values for the split separating most of the Mediterranean specimens from Greater Caribbean and Sao Tome specimens were high (NJ Bootstrap = 0.99, ML bootstrap = 98%, BI posterior probability = 100%). Additionally, high support was given to a subgroup containing 11 specimens from St. Peter and St. Paul Archipelagos (ML bootstrap = 87, BI posterior probability = 100). Two specimens collected from Malta and Crete clustered with Greater Caribbean individuals occupying distal branches in all three analyses, indicating a close relationship to more recent lineages in that region. However, support values for these topologies are low, with the exception of high Bayesian posterior probability (90%) for a branch containing the specimen from Malta. A third phylogenetic outlier from Crete was placed in a basal cluster within the predominantly Greater Caribbean clade that also received low support from each analysis (NJ Bootstrap = 0.32, ML bootstrap = 40%, BI posterior probability = 60%).

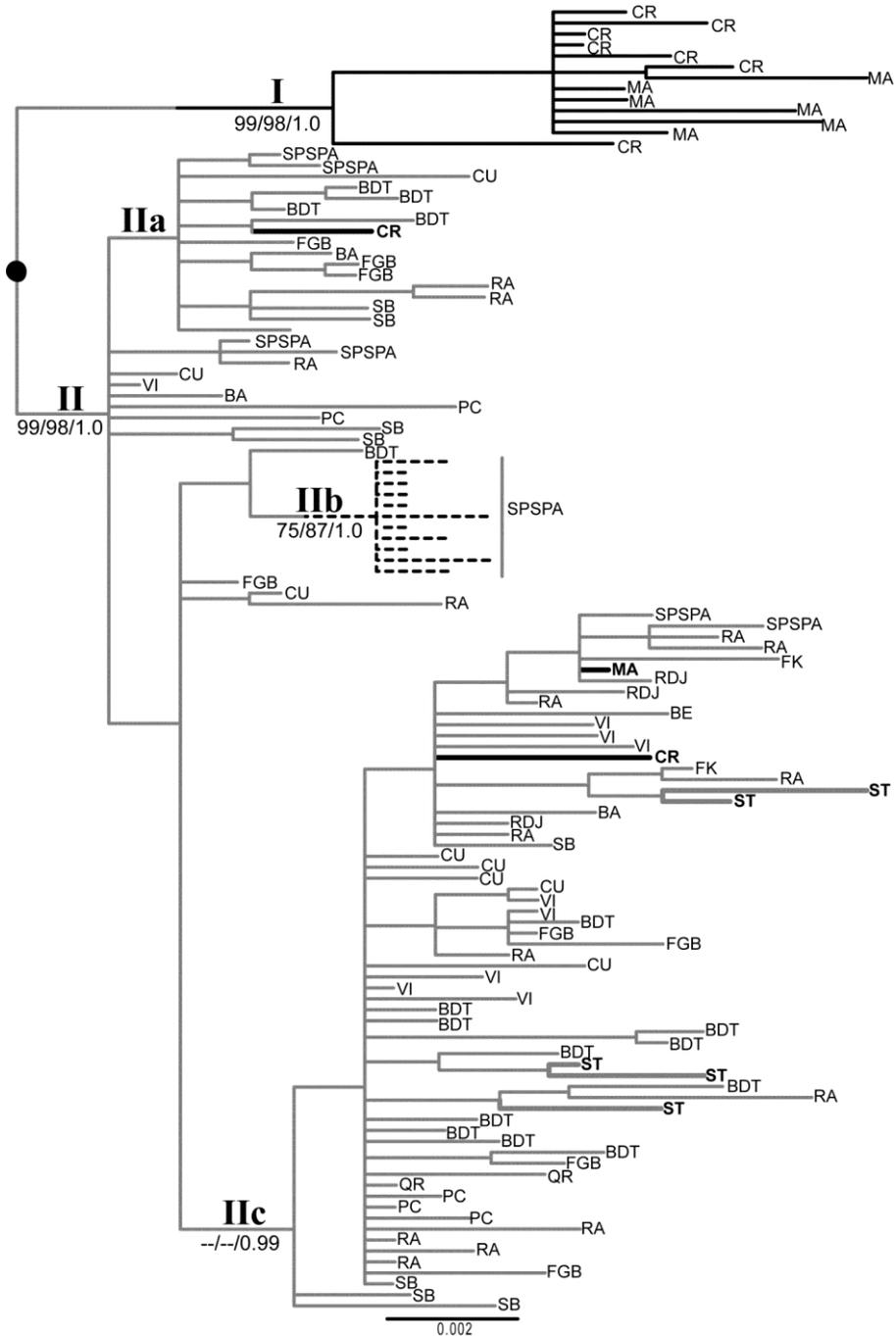


Fig. 1 50% majority-rule consensus tree constructed using Bayesian Inference. Scale bar indicates the number of substitutions per site. Branch support values (Neighbor-Joining/ Maximum Likelihood/ Bayesian Inference) are displayed for clades recovered by all three analysis methods. For a list of locale abbreviations used in the tree, refer to Table 1.

Genetic Diversity

Corrected average genetic distances among *Hermodice* populations based on COI sequence data (Table 2) ranged from 0.90% (Rocas Atoll vs. U.S. Virgin Islands: SD = 0.18%) to 2.92% (Sao Tome vs. Crete: SD = 0.57% and Sao Tome vs. Malta: SD = 0.56%). Distances between non-Mediterranean populations ($0.83\% \pm 0.17\%$ to $1.54\% \pm 0.34\%$) were consistently lower than those between Mediterranean and non-Mediterranean populations ($2.11\% \pm 0.43\%$ to $2.92\% \pm 0.57\%$). Similarly, low average genetic distances between Mediterranean and non-Mediterranean populations were observed with ITS data ($1.64\% \pm 0.46\%$).

Analysis of molecular variance for COI indicated somewhat small but statistically significant separation between Eastern and Western Atlantic populations ($\Phi_{st} = 0.51$; p-value < 0.00001) with 38.44% of variance occurring among the two Atlantic groups, 12.09% between the populations within groups, and 49.46% within populations. This difference was greater ($\Phi_{st} = 0.59$; p-value < 0.00001) when comparing Mediterranean vs. Atlantic populations (53.76% of total variance) in a separate analysis (4.93% among populations within groups, 41.31% within populations). Lower but still significant differences were observed between Eastern and Western Atlantic populations ($\Phi_{st} = 0.40$; p-value < 0.00001 with 31.39% of variance among groups, 8.95% among populations within groups, and 59.65% within populations) and between Mediterranean vs. Atlantic populations ($\Phi_{st} = 0.47$; p-value < 0.00001 with 45.93% of variance among groups, 4.16% among populations within groups, and 49.91% within populations) for 16s rDNA sequence data as well.

Table 2 Mean corrected genetic distances (COI) between (lower diagonal) and within (diagonal) selected groups. The numbers in each column correspond to the numbered sample sites in each row. Values reflect the estimated number of substitutions per site according to Kimura's 2-parameter model. Standard errors of genetic distances (upper diagonal) are also included

	1	2	3	4	5	6	7	8	9	10	11
1. St. Peter and St. Paul Archipelago	0.0074	0.0027	0.0030	0.0028	0.0053	0.0029	0.0027	0.0053	0.0028	0.0028	0.0034
2. Curacao	0.0114	0.0097	0.0017	0.0019	0.0050	0.0023	0.0019	0.0050	0.0019	0.0021	0.0025
3. U.S. Virgin Islands	0.0116	0.0083	0.0073	0.0027	0.0053	0.0023	0.0019	0.0052	0.0018	0.0020	0.0023
4. Bocas del Toro	0.0122	0.0102	0.0095	0.0106	0.0049	0.0023	0.0021	0.0050	0.0020	0.0021	0.0026
5. Crete	0.0248	0.0240	0.0251	0.0247	0.0115	0.0043	0.0052	0.0020	0.0050	0.0050	0.0057
6. Flower Garden Banks	0.0134	0.0116	0.0111	0.0121	0.0211	0.0129	0.0023	0.0043	0.0023	0.0022	0.0030
7. Panama City FL	0.0115	0.0097	0.0093	0.0107	0.0252	0.0122	0.0104	0.0051	0.0020	0.0019	0.0027
8. Malta	0.0256	0.0247	0.0255	0.0255	0.0129	0.0219	0.0255	0.0156	0.0050	0.0050	0.0056
9. Rocas Atoll	0.0117	0.0097	0.0090	0.0106	0.0248	0.0122	0.0105	0.0252	0.0102	0.0021	0.0025
10. Sonnier Bank	0.0116	0.0098	0.0092	0.0106	0.0242	0.0116	0.0092	0.0246	0.0104	0.0098	0.0027
11. Sao Tome	0.0154	0.0121	0.0108	0.0128	0.0292	0.0152	0.0132	0.0292	0.0124	0.0133	0.0123

Morphological Analysis

The number of branchial filaments at the 10th chaetiger ranged from 8 to 54 (Mean: 18.4) for East Atlantic fireworms and from 18 to 137 (Mean: 58.8) in West Atlantic fireworms. Outputs for all variables tested (number of filaments at 10th chaetiger, filaments at 10th chaetiger/number of chaetigers, and average number of medial filaments/ number of chaetigers) were approximately normally distributed. Variances of filament abundance in the East and West Atlantic samples were significantly unequal according to Brown-Forsythe statistics (p-values: 0.002 – 0.02). Comparisons of means via the Student's t-test indicated differences of 40.3 branchial filaments at the 10th chaetiger (Wilcoxon $p < 0.0001$), 0.53 for the number of 10th segment filaments/ number of chaetigers (Wilcoxon $p < 0.0001$) and 0.50 for average number of medial filaments/ number of chaetigers (Wilcoxon $p < 0.0001$) (Figure 2).

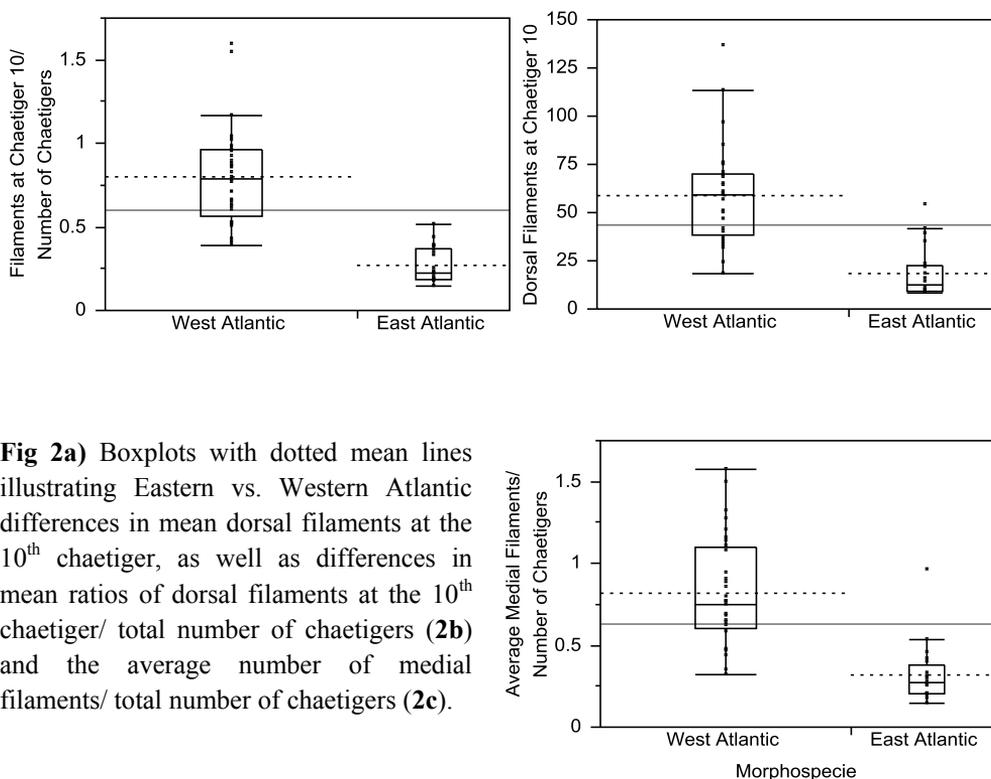


Fig 2a) Boxplots with dotted mean lines illustrating Eastern vs. Western Atlantic differences in mean dorsal filaments at the 10th chaetiger, as well as differences in mean ratios of dorsal filaments at the 10th chaetiger/ total number of chaetigers (**2b**) and the average number of medial filaments/ total number of chaetigers (**2c**).

A logistic regression based on the number of filaments at the 10th chaetiger/number of chaetigers (Generalized $R^2 = 0.88$) for all 53 specimens analyzed calculated high probabilities (> 0.99) that the two phylogenetic outliers from Crete, which were too small to be properly scored according to previously established criteria, belong to the East Atlantic population. In contrast, the phylogenetic outlier from Malta, with 52 dorsal branchial filaments at the 10th chaetiger, received relatively low probability (0.55) for belonging to the West Atlantic population, along with the specimen from Sao Tomé ($P = 0.88$). Overall, the logistic regression using the aforementioned ratio was 92.45% compatible with the original diagnosis of the specimens studied, which was based solely on location; in both groups, the regression assigned 2 specimens to a morphospecies not purported to inhabit the location in which they were collected. Power regression analysis (Figure 3) produced trend lines with a good fit ($R^2 = 0.833$), but the interaction between region (East vs. West Atlantic) and filament abundance was not statistically significant at the $\alpha = 0.05$ level ($p = 0.142$).

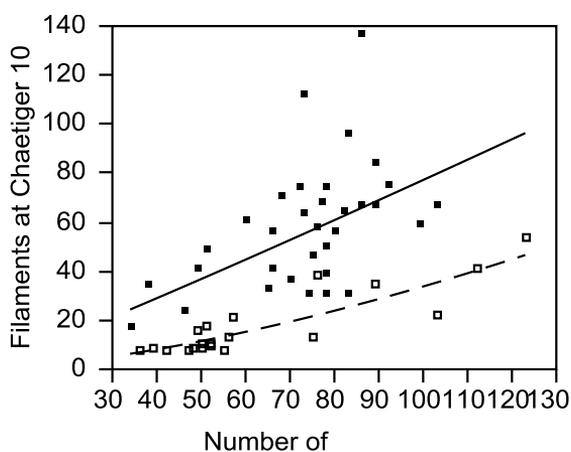


Fig. 3 Power regression illustrating the relationship between dorsal branchial filament abundance at chaetiger 10 and the total number of chaetigers for Eastern Atlantic (hollow squares with dashed trend line) and Western Atlantic (solid squares with solid trend line) bearded fireworms. Note that the interaction between morphology and region (the existence of the two separate trend lines) is not statistically significant.

Discussion

Many studies measuring the divergence of neutral mitochondrial markers such as COI and 16srDNA between polychaete congeners over a broad oceanographic range, including cases of cryptic speciation, have reported relatively high genetic distances among the populations in question. Phylogenetic analysis of COI and 16srDNA for *Palola* has revealed deep divergences among genetic lineages (Schulze 2006) and similar patterns have been observed for congeners within *Diopatra* (Pires et al., 2010), *Streblospio* (Schulze et al., 2000), and *Owenia* and *Pectinaria* (Jolly et al., 2006). Within amphinomids, cryptic species in the fireworm genus *Eurythoe* occurring in the Atlantic were found to be about 10% divergent (COI), indicating a significant period of isolation between coastal/island and island-restricted specimens (Barroso et al., 2010). By contrast, the most divergent *Hermodice* populations in our study differ by less than 3.0% in terms of corrected COI sequence data. Ironically, the largest divergence detected in our study (2.9%) was between populations in Sao Tomé and the Mediterranean Sea despite the recent reinstatement of these populations as a single species based on morphological data (Yáñez-Rivera and Salazar-Vallejo, 2011). Furthermore, all non-Mediterranean specimens examined appear to belong to a single, large clade containing a multitude of individual haplotypes.

The private lineage found in the Mediterranean Sea (Clade I) may have arisen during one of the glaciation events in the Pleistocene, which have been implicated in the development of phylogenetic patterns of other fauna in the region (Alvarado Bremer et al., 2005; Viñas et al., 2004). Calibrated molecular clocks for COI applied to polychaetes range from about 2%/Ma (Hickerson et al., 2003) to 2.2%/Ma (Chevaldonné et al., 2002) to 7%/Ma in amphinomids (Barroso et al., 2010). This places the origin of the Atlantic-Mediterranean divergence between the Donau Glaciation of the early Calabrian (1.5Ma) and the Riss Glaciation roughly 150,000 years ago (Emig and Geistdoerfer, 2004; Laubier and Emig, 1993).

Presently, though, Clade I shares its range with lineages from elsewhere in the Atlantic Ocean. Because some of the outliers in the phylogenetic analyses occupy distal branches in the Atlantic clade, the reinvasion of the Mediterranean might have been very recent. Generally speaking, there are two possible explanations for the presence of Atlantic lineages in the Mediterranean. On one hand, natural but rare immigration from the Atlantic might be accomplished by mature adults via oceanic rafting and/or by planktonic transport of teleplanic larvae. Gene flow in these natural scenarios is expected to be unidirectional because the Mediterranean Sea experiences a virtually constant inward flux of surface water from the Atlantic Ocean through the Strait of Gibraltar (Bryden, 1990; Bryden et al., 1994), and this expectation is corroborated by our results. On the other hand, the introduction of foreign populations into Mediterranean waters may have been caused by anthropogenic activity, although the usually suspected mechanisms of transport such as ballast water (Bastrop and Blank, 2006; Blank et al., 2007) and hull fouling (Farrapeira et al., 2007) do not account for unidirectional gene flow.

However, not all anthropogenic transport mechanisms are inherently bidirectional. For instance, when the Monaco Oceanographic Museum inadvertently released the invasive macroalgae *Caulerpa taxifolia* into Mediterranean waters in 1984, it was found during the same year in Southern California, and later discovered in areas along the Australian Coast (Jousson et al., 2000; Schaffelke et al., 2002). Eventually, molecular analysis confirmed that this strain of *C. taxifolia* originated from Moreton Bay in Australia, and its dispersal to California and other parts of Australia was facilitated by the aquarium trade (Jousson et al., 2000; Meusnier et al., 2001; Meusnier et al., 2002; Meusnier et al., 2004; Walters et al., 2006; Wiedenmann et al., 2001). One of the proposed mechanisms of introduction in these areas was aquarium dumping of imported coral rubble known as “live rock” along local coastlines (Stam et al., 2006). Because bearded fireworms are corallivores (Lewis, 2009), they utilize reef structures such as coral rubble for shelter and often hide in cracks and crevices on reef rocks. Moreover, they are associated with reef habitats in the Florida Keys and Caribbean where “live rock” is cultured and

harvested. Therefore, it is possible that the individuals from Clade II found in the Mediterranean are the result of aquarium dumping by recreational aquarists, a practice that has led to the introduction of exotic species along the American coast as well (Semmens et al., 2004).

The unique phylogenetic signal observed from Saint Peter and Saint Paul Archipelago, wherein the majority of individuals belong to a private and highly supported subgroup within Clade II, suggests that a large portion of the population arose from a single colonization event, though migrants from other locales have separately colonized the region as well. Alternatively, the subgroup may be an artifact of a past selective sweep followed by mutation at neutral sites and subsequent reinvasions. In either case, the small size of the archipelago and its considerable distance from coastal habitats (about 600 miles from Brazil) probably facilitated the domination of this region by a single lineage. This situation can be contrasted with Rocas Atoll, a similarly small landmass examined in this study that apparently does not retain a unique phylogenetic signal, probably because of its closer proximity to neighboring landmasses.

Previous researchers reported an allometric relationship between branchial filament abundance/ total number of chaetigers in East vs. West Atlantic populations (Yáñez-Rivera and Salazar-Vallejo, 2011). While our analyses indicate that East Atlantic bearded fireworms tend to have fewer branchial filaments per chaetiger than those in the West Atlantic, a statistically significant interaction between filament abundance and region has not been indicated for the specimens examined. Furthermore, the molecular markers we have tested do not indicate a genetic basis for this particular character, as phylogenetic outliers in the Mediterranean can still exhibit the reduced branchial filament abundance typical for the region. If instead, the regional difference in filament abundance is a result of phenotypic plasticity, it may be caused by regional differences in oxygen availability. The Mediterranean Sea, where bearded fireworms tend to have fewer filaments, is more oxygenated on average than other parts of the Atlantic Ocean (Figure 4). Since more branchial filaments provide a larger surface area for gas exchange, individuals that have more

filaments and live in warmer, less oxygenated waters would be better adapted to their environment. This kind of change in filament abundance in response to oxygen availability has been demonstrated for spionid polychaetes (Lamont and Gage, 2000).

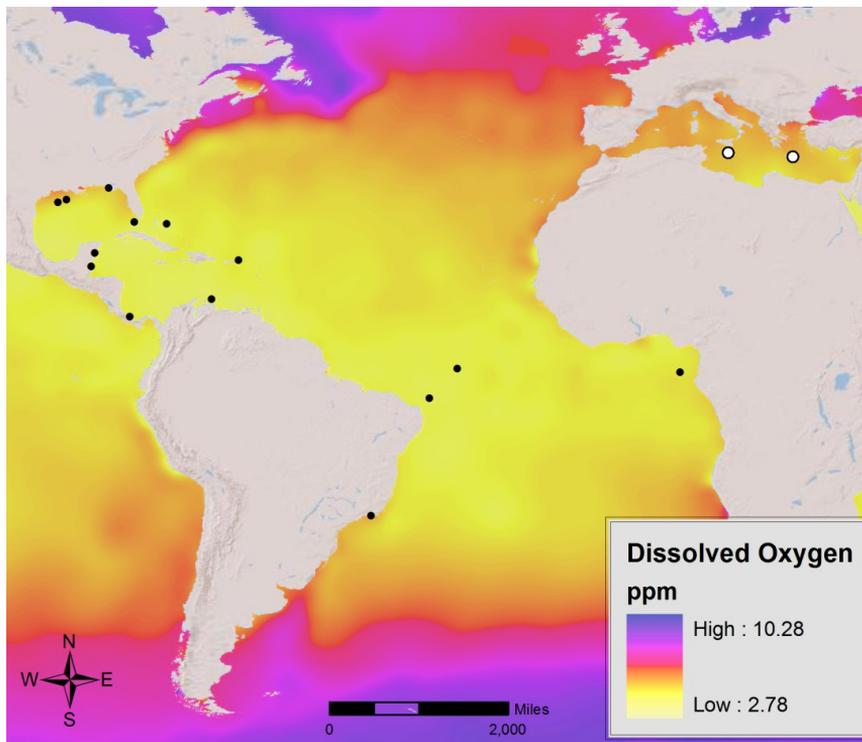


Fig 4 Black and white dots indicate collection sites. White dots indicate locations where the private lineage (Clade I) was discovered. World oxygen data was taken from BioOracle (2009).

It is not clear whether the morphological differences observed in bearded fireworms are the result of local adaptation (genetic) or phenotypic plasticity (environmental) because, although there does not appear to be a link between morphology and the neutral markers examined, a strong selective gradient may still be acting on the genes responsible for morphology (Conover et al., 2006). Addressing this issue would require whole genome data and a series of either common garden or reciprocal transplant studies. Moreover, a much better

understanding of the reproductive biology of *Hermodice* is needed for studies that could detect selective gradients and genetic/environmental interactions. Conversely, what is clear from the results of this study is that a) the range of *H. nigrolineata* proposed by previous research is not supported by molecular data, b) COI divergences between the Mediterranean and Atlantic clades are low compared to other polychaete genera and occasional gene flow occurs, and c) it is unlikely that phylogenetic outliers in the Mediterranean sea, especially under 50 chaetigers, could be reliably distinguished from those in the private lineage via comparison of branchial filaments. Hence, bearded fireworms represent an unusual case among polychaetes that were previously considered cosmopolitan. Whereas molecular studies of fireworms and other polychaetes have typically revealed cryptic speciation events and deep divergences between genetic lineages, our study indicates a high degree of relatedness and population connectivity for *H. carunculata* throughout the Atlantic Ocean.

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3

Contact with macroalgae causes variable coral mortality in *Montastraea faveolata*

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Abstract

Shifts in benthic reef community structure often involve the replacement of corals by macroalgae. We investigated the response of a scleractinian coral to direct contact with different macroalgae during an *in situ* interaction experiment on Curaçao, southern Caribbean. The macroalgae *Dictyota pinnatifida*, *Lobophora variegata*, *Halimeda opuntia* and *Cladophora* spp. were placed onto healthy *Montastraea faveolata* colonies and coral condition was monitored over a period of 15 d. Rapid coral tissue mortality was observed in colonies interacting with *D. pinnatifida* and *Cladophora* spp. In contrast, mortality in the *H. opuntia* and *L. variegata* treatments appeared slowly. At day 3, coral tissue in contact with *D. pinnatifida* and *Cladophora* spp. experienced 55 and 71% mortality, respectively, whereas mortality remained less than 2 % in the *H. opuntia* and *L. variegata* treatments. At day 15, mortality reached 38 % in the *L. variegata* treatment, whereas all other algae caused ≥ 88 % coral mortality. All algae except *L. variegata* caused mortality outside the area overgrown by the transplants, suggesting white plague disease-like processes beyond the area of direct interaction. Such differential coral mortality could be attributed to variable algal-induced exudation of allelochemicals and/or DOC release rates by means of direct toxicity or by fueling microbial activity with ensuing oxygen deficiency.

Introduction

In the Caribbean, the average cover of hard corals has declined by ~ 80 % in the last 30 years (Gardner et al. 2003). Reef degradation often involves a shift in benthic community structure in which corals are replaced by macroalgae (Hughes 1994). Such changes may be initiated by a combination of several factors such as climate change, over-fishing and human-derived pollution (Hughes et al. 2007), bleaching events (Diaz-Pulido and McCook 2002) and the increase of coral disease outbreaks (Rosenberg and Ben-Haim 2002; Sutherland et al. 2004). However, the extent to which macroalgae drive this shift by outcompeting scleractinian corals is still uncertain (Aronson and Precht 2006; Diaz-Pulido et al. 2009).

Studies investigating the interaction between macroalgae and scleractinian corals provide evidence for: (i) a decrease in fecundity and growth rates of scleractinian corals interacting with algae (Box and Mumby 2007; Birrell et al. 2008; Foster et al. 2008); (ii) negative effects of nutrients on coral-algal competition (Szmant 2002; Vermeij et al. 2010); (iii) negative microbial (Smith et al. 2006); and (iv) allelochemical effects of macroalgae on corals (Rasher and Hay 2010; Paul et al. 2011; Rasher et al. 2011). In the southern Caribbean, the macroalgae *Halimeda*, *Cladophora*, *Lobophora* and *Dictyota* constitute common and increasingly abundant genera (Nugues and Bak 2008). These algae can impact scleractinian corals via lipid-soluble metabolites transferred via direct contact (Rasher and Hay 2010) or by triggering coral diseases (Nugues et al. 2004). Several recent studies also support that these algae can release dissolved organic carbon (DOC) which in turn affect microbial activity at the coral-algal interface and result in coral death (Wild et al. 2008; Barott et al. 2011).

Here, we describe results from an *in situ* interaction experiment on a coral reef adjacent to Curaçao, southern Caribbean, designed to characterize the species-specific impact of macroalgae on coral health. The observed patterns were discussed in the light of different processes potentially involved in coral-algal interactions.

Studies evaluating specific algal characteristics and/or effects help to identify the mechanisms underlying coral-algal interactions and can provide further knowledge to understand the causation and to improve the management of algal occurrences on Caribbean coral reefs.

Material and Methods

The experiment was carried out in December 2011 at Carmabi Buoy Zero (12°07'N, 69°57'W) on Curaçao, Southern Caribbean. The following macroalgae were used: *Dictyota pinnatifida*, *Lobophora variegata*, *Halimeda opuntia* and *Cladophora* spp. All macroalgae were collected at the experimental site at 5 - 12 m water depth using SCUBA. Back in the laboratory, they were cleaned from epiphytes and invertebrates. Aliquots of algae (70 g wet weight) were placed in small flexible mesh bags (made of nylon fishing nets, 1 cm mesh size) and deployed back at the experimental site onto healthy colonies of the coral *Montastraea faveolata* (n = 5 colonies per algal species) at 6 - 8 m water depth (Fig. 1). Due to the large mesh size and thin structure, nets were assumed not to interfere with flow and fluxes of algal exudates. Nets were attached to rock substrate adjacent to the coral tissue to avoid tissue damage. The health of the coral tissue interacting with the algae was monitored on a daily basis for the first 6 d and thereafter on days 10 and 15 by temporarily removing the nets during the monitoring. Coral condition was differentiated by using healthy, bleached and dead tissue as categories. Tissue which showed a change in coloration only was considered bleached, whereas the dead condition was characterized by decaying and sloughing of tissue. The surface area for each tissue category under the net was measured to the nearest centimeter using a transparent grid (1 cm²) and expressed as a percentage of the total area overgrown by algae.

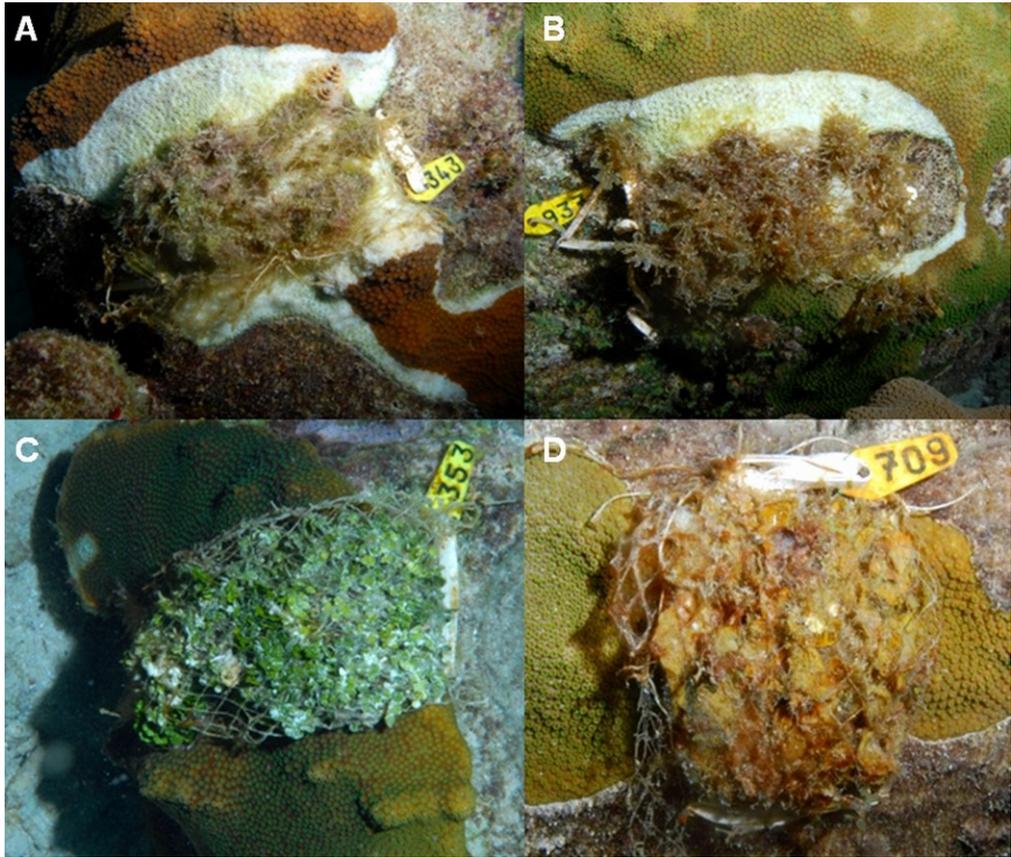


Fig. 1 Nets with macroalgae *Cladophora* spp., *Dictyota pinnatifida*, *Halimeda opuntia* and *Lobophora variegata* (A-D) deployed on *Montastraea faveolata* colonies.

In cases of exceptional tissue degradation beyond the area of direct interaction, the surface areas of dead tissue were also estimated outside the nets using the transparent grid. In addition, the presence of coral derived mesenterial filaments was recorded on a semi-quantitative scale.

Earlier experimental work at the same study sites showed that strict controls (no algae and nets) did not produce any mortality (Nugues et al. 2004). During a previous *in situ* experiment, we deployed 20 identical empty mesh bags as procedural controls on *M. faveolata* corals on the identical site at Buoy Zero. Over 25 days, no or little (< 5 % dead tissue) coral mortality and bleaching on colonies in

contact with empty nets only was observed (A. Wolf unpubl. data). Therefore, due to the similarity of the experiments, controls were not used during this experiment.

Repeated measure ANOVAs were used to assess differences among algal species in coral mortality over time. Lack of homogeneity in variances was corrected by log-transformation of the data prior to analysis, as confirmed by Levene's test. All analysis used sphericity correction according to Greenhouse-Geisser and Huynh-Feldt-Lecoutre. To determine differences among specific treatments and time intervals, Tukey's post-hoc test (HSD) was applied.

Results

Rapid tissue deterioration was observed for corals interacting with *D. pinnatifida* and *Cladophora* spp. (Fig. 2A-B). After 3 d of contact, colonies showed 55 ± 17 % (means \pm SE) and 71 ± 6 % dead tissue, respectively. In contrast, colonies interacting with *H. opuntia* and *L. variegata* displayed a much slower onset of coral mortality, yet a longer and more pronounced bleaching state, and both treatments caused less than 2 % dead tissue on day 3 (Fig. 2C-D). Over the next 7 d, tissue mortality reached close to 100 % in the *D. pinnatifida* and *Cladophora* spp. treatments and coral bleaching never reached more than 40 % in colonies contacting these algae. *Cladophora* spp. caused the most rapid increase in tissue death and also displayed the shortest period of bleaching (3 d in total) among all algae. In the *H. opuntia* and *L. variegata* treatments, colonies showed 48 ± 13 % and 58 ± 15 % bleached tissue on day 6 and both treatments induced bleaching for a period of 13 days in total. After 10 and 15 days, the *H. opuntia* treatment reached coral mortality rates of 68 ± 15 % and 88 ± 12 %, respectively. In contrast, *L. variegata* treatments had the lowest rates of coral mortality, i.e. 10 ± 9 % and 38 ± 18 % on days 10 and 15, respectively. Furthermore, *L. variegata* induced coral bleaching up to 74 ± 18 % on day 10.

● Healthy tissue ○ Bleached tissue ▼ Dead tissue ★ Dead tissue outside algal nets

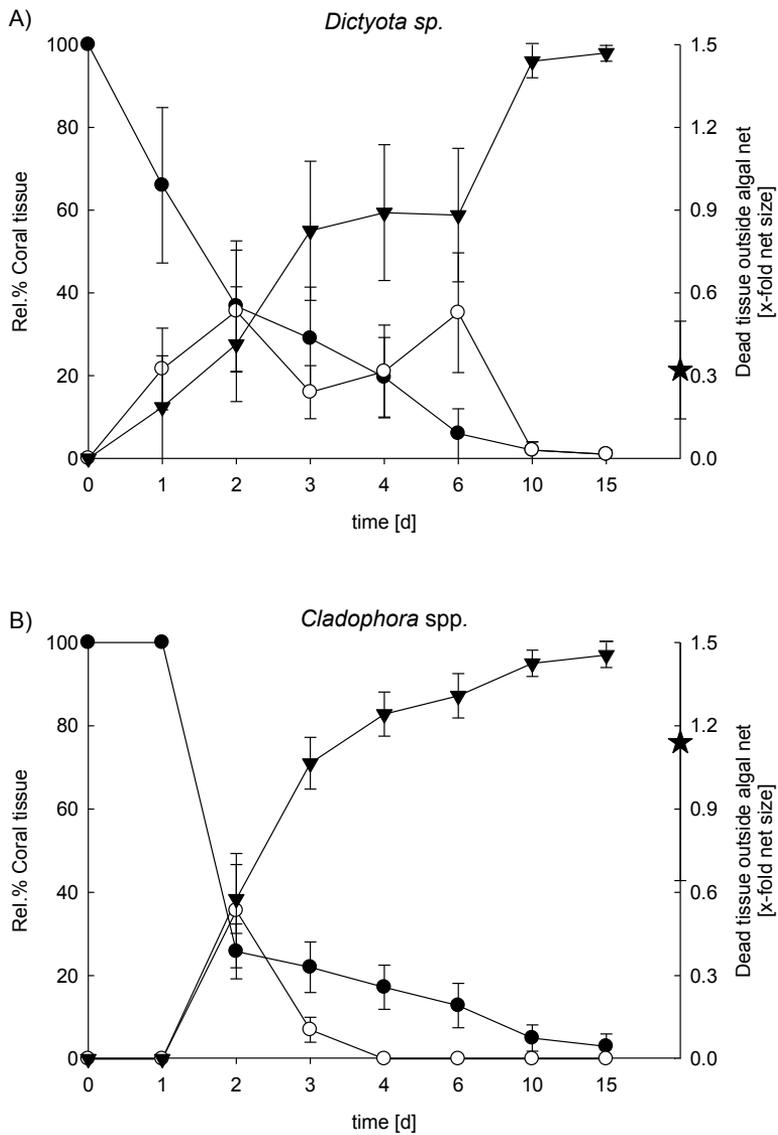


Fig. 2 A-B) Changes in coral health categories (healthy, bleached and dead tissue; \pm SE; $n = 5$) in the different algal treatments. Tissue loss outside areas of direct coral-algal interaction (2nd y-axis) is displayed as x-fold algal net size (\pm SE).

● Healthy tissue ○ Bleached tissue ▼ Dead tissue ★ Dead tissue outside algal nets

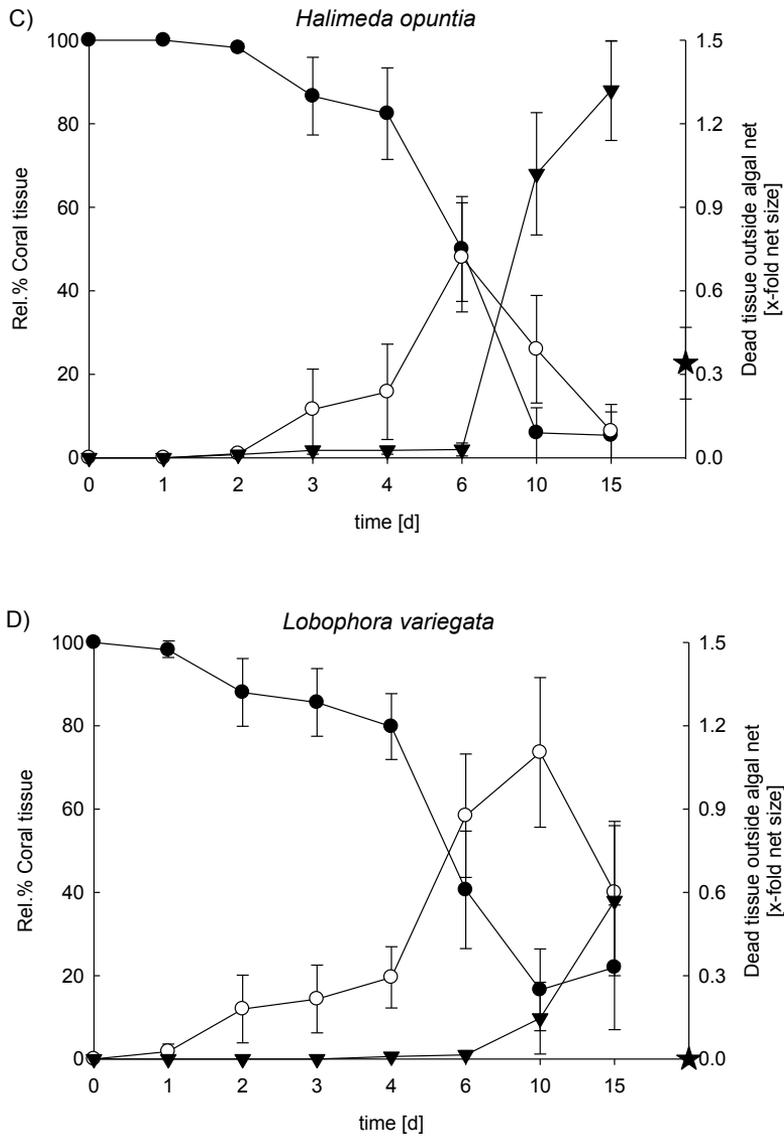


Fig. 2 C-D) Changes in coral health categories (healthy, bleached and dead tissue; \pm SE; $n = 5$) in the different algal treatments. Tissue loss outside areas of direct coral-algal interaction (2nd y-axis) is displayed as x-fold algal net size (\pm SE).

Coral mortality outside the area overgrown by the algal transplants was observed for all algae except *L. variegata* (Fig. 1 & 2). The highest value was observed for *Cladophora* spp., which caused a total coral mortality more than twice the size of the algal overgrown area. Mesenterial filaments (MF) were observed from the initiation of the interaction until coral death, with an increase in the number of filaments just prior to coral death (Table 1).

Table 1 Abundance of coral derived mesenterial filaments (MF) over time (day 1-15) for each algal treatment. (-) MF absent, (+) few (1-5) MF, (++) many (>5) MF present per replicate.

<i>M.faveolata</i> colony vs.	1	2	3	4	6	10	15
<i>D. pinnatifida</i>	++	-	-	-	-	-	-
<i>Cladophora</i> spp.	++	+	-	-	-	-	-
<i>H. opuntia</i>	+	+	+	+	++	+	-
<i>L. variegata</i>	+	+	+	+	+	+	+

Differences in coral mortality among algal treatments were statistically significant (Table 2). *D. pinnatifida* and *Cladophora* spp. caused significantly higher coral mortality compared to *H. opuntia* and *L. variegata* on days 2 to 6. On days 10 and 15, mortality was less in *L. variegata* compared to all other algal species.

Table 2 GLM Repeated measure ANOVA with algal species as between-factor variable and day as within-factor variable, followed by post-hoc multiple comparisons for each algal treatment.

	Df	Type I SS	MS	F	P
Algal species	3	622.3	207.4	28.0	<.0001
Day	6	674.4	112.4	68.1	<.0001 ^a
Day*Algal species	18	204.7	11.4	6.9	<.0001 ^a

^a $P < 0.0001$ with/without using the sphericity correction factors ϵ_1 (Greenhouse-Geisser) or ϵ_2 (Huynh-Feldt-Lecoutre)

Tukey's post-hoc tests (HSD)

Algal species/Day	0	1	2	3	4	6	10	15
<i>D. pinnatifida</i>	A	A	A	A	A	A	A	A
<i>Cladophora</i> spp.	A	A	A	A	A	A	A	A
<i>H. opuntia</i>	A	A	B	B	B	B	A	A
<i>L. variegata</i>	A	A	B	B	B	B	B	B

Discussion

A number of physical and chemical factors can interact to cause the observed rapid decline of coral health. These include physical abrasion and shading through direct contact between algae and corals (Lirman 2001), mucus production and microbial growth on the coral-algal boundary layer, which in turn may be stimulated by primary or secondary metabolites released by algae (Wild et al. 2008; Rasher and Hay 2010).

An increase in coral mucus will stimulate bacterial growth on the coral surface mucus layer (Segel and Ducklow 1982). A healthy coral holobiont normally actively controls the growth rate of its associated microbes and favors bacteria with a protective role against pathogens by occupying entry niches, or with antibiotic functionality against pathogens (Ritchie 2006). Contact with algae could impair this functionality, resulting in coral pathologies and mortality.

Barrot et al. (2011) carried out direct interaction experiments with *Montastraea annularis*, using *H. opuntia*, *Dictyota bartayresiana* and coral turf assemblages. Coral-associated bacterial density and diversity increased significantly for *D. bartayresiana*, but not for *H. opuntia* interactions. Principal component analysis also showed close clustering for coral- and *H. opuntia* derived bacteria, compared to a distant clustering for *D. bartayresiana* derived bacteria. Comparably, our study showed strong initial differences in coral mortality between the *H. opuntia* and *D. pinnatifida* treatments, the latter one causing very rapid tissue degradation compared to *H. opuntia* treatments. Yet, at the end of the experiment, *H. opuntia* and *D. pinnatifida* produced similar effects on coral mortality. It is plausible that the rapid coral mortality produced by *Dictyota* sp. was caused by the high dissimilarity in bacterial clades between the macroalgae and the corals. At present, the role of algal-derived bacteria in coral-algal interactions is unclear.

Rasher and Hay (2010) found macroalgae-derived secondary metabolites to cause bleaching and mortality in scleractinian corals. In their 20-d assays, lipid-

soluble extracts, either excretable or present on the algae's exterior, of *D. bartayresiana*, *H. opuntia* and *L. variegata* also caused a significant, but comparable decrease in photosynthetic activity in *Porites porites*, underlining their potential to poison corals upon direct contact. Interestingly, direct macroalgal contact revealed differences between *D. bartayresiana* and the other two macroalgae in their abilities to suppress photosynthetic activity, lending support for additional, e.g. primary metabolite-induced effects such as DOC release. During our 15-d experiment, *L. variegata* had a lower impact on coral mortality compared with *D. pinnatifida* and *H. opuntia* and *D. pinnatifida* showed a strong initiation of coral mortality. These differences could also be associated with differing release speed of secondary metabolites among algae and/or different allelopathic activity of the same alga in time and space depending on e.g. grazing pressure or nutrients (Paul and Fenical 1986).

Under laboratory conditions, Smith et al. (2006) proved that algal-derived, primary metabolites such as dissolved organic carbon can lead to coral mortality. DOC can increase microbial growth by an order of magnitude, suggesting coral-associated microbes to be carbon-limited (Kline et al. 2006). A disrupted coral-microbe relationship due to organic carbon loading (e.g. glucose) could thus directly cause coral mortality by over-stimulating growth of coral mucus-associated microbes. However, these laboratory-based results have yet to be demonstrated *in situ*. The lack of comparative studies on DOC release rates of the investigated macroalgae also precludes comparisons between DOC rates and algal-generated coral mortality. Much of the mucus-associated carbon remains in a refractory form which is not available for microbial growth (Herndl and Velimirov 1986; Wild et al. 2004). *H. opuntia* associated DOC was found to consist of 59 % carbohydrates (Haas and Wild 2010). Haas et al. (2011) showed that *H. opuntia* is releasing high-quality DOC which can be efficiently metabolized by microbes. Therefore even small amounts of DOC could alter microbes in the surface mucus layer (SML) and subsequent coral health. The increase of algal induced DOC in the form of simple

sugars may enable microbes to break down more complex and previously unavailable carbon sources via co-metabolism (Dinsdale et al. 2008).

Although anaerobic conditions occur during coral-algal interactions, hypoxia, considered a fluctuating stressor by Wangpraseurt et al. (2012), is unlikely to cause coral mortality by itself. *H. opuntia* was found to rarely and reversibly (after withdrawal) induce hypoxia at the surface boundary layer upon direct contact with *M. annularis*, compared to a more pronounced and partly irreversible hypoxic effect by *D. bartayresiana* (Barott et al. 2009; 2011). Under aerobic conditions, increased DOC availability can fuel bacterial growth by enabling rapid aerobic respiration. A shift towards prolonged anaerobic conditions may facilitate additional fermentative processes by anaerobes. Both metabolic pathways can be fueled by excessive DOC release, thus leading to the observed differences in coral mortality.

Organic carbon treatments can cause pathologies similar to those reported for band diseases, including rapid sloughing of coral tissue (Kuntz et al. 2005). Tissue death occurring outside the area overgrown by our algal transplants was similar to white plague type II like disease signs and lacked any state of bleaching prior to mortality. Effects of algal metabolites have been shown to be restricted to the diffuse boundary layer and to extend only within a few millimeters away from the coral-algal interface in natural conditions (Wangpraseurt et al. 2012). In addition, Pantos et al. (2003) demonstrated a whole-animal response to disease with shifts in the microbial community of even healthy looking tissue. Although metabolites from macroalgae may have more extensive effects, it is likely that coral mortality beyond the area of algal overgrowth, though initially triggered by algal exudates, was mediated by microbes.

Together our findings suggest that macroalgae cause differential mortality in scleractinian corals. These differences are likely to be associated with a variety of algal characteristics and to depend on the magnitude by which these properties interact to eventually initiate a cascade of microbial processes leading to the observed differences.

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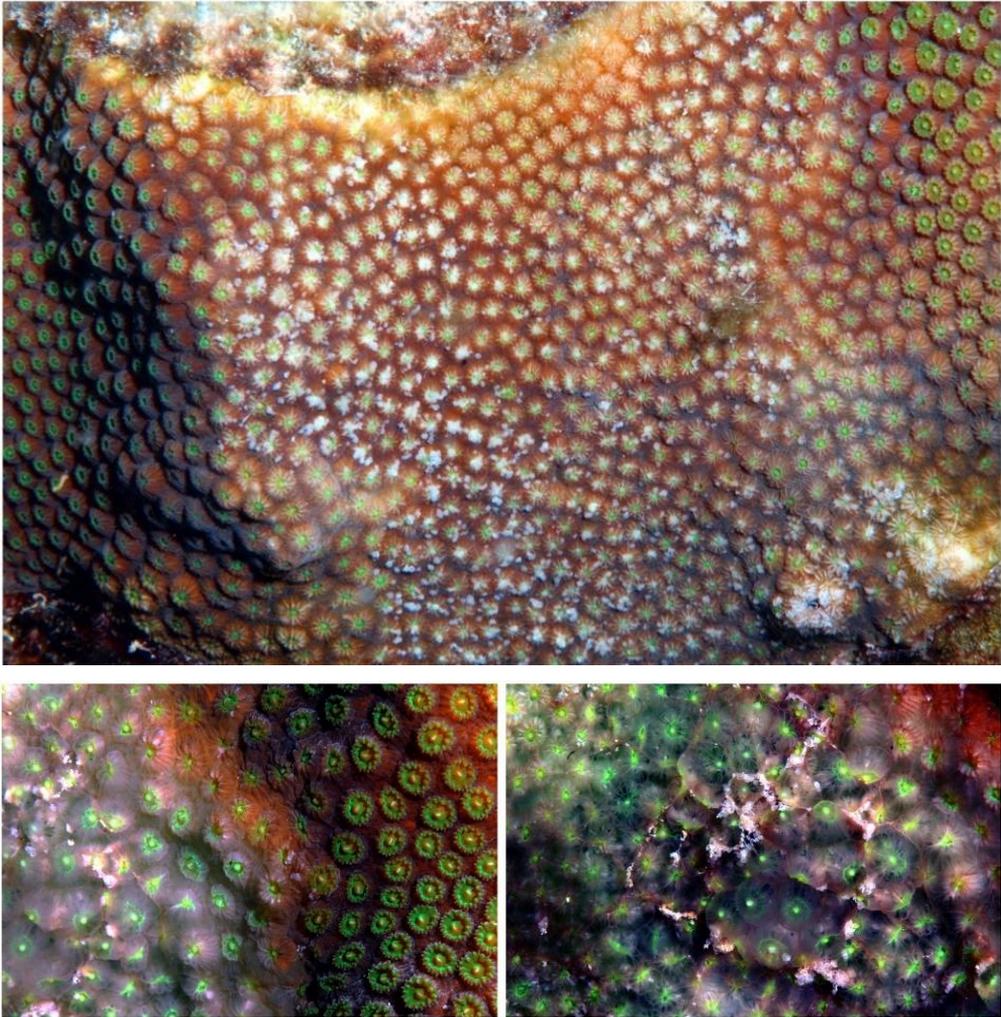
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Appendix 1



Above: Mesenterial filaments extruding on the surface of *Montastraea faveolata* after 1 d of direct contact with the macroalgae *Dictyota pinnatifida*

Below: Degradation of *Montastraea faveolata* polyps. Exposure to macroalgae can lead to “blown up” coral polyps prior to sloughing off of coral tissue.

4

Predation on coral settlers by the corallivorous fireworm *Hermodice carunculata*

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Abstract

Coral predation by the fireworm *Hermodice carunculata* was investigated by presenting settlers (< 3-weeks-old) and adults of two species of Caribbean corals, *Montastraea faveolata* and *Agaricia humilis*, to three different size classes of fireworms under laboratory conditions. For both coral species, survival rates of settlers were low (< 2% after 4 days), intermediate (42-54%) and high (> 90%) in the presence of small-, mid- and large-sized fireworms, respectively. In contrast, fireworms hardly predated on adult corals, irrespective of their sizes. Our results suggest an ontogenic shift in the diet of *H. carunculata* and in the susceptibility of corals to predation by fireworms. *H. carunculata*, in particular small-sized individuals, could be an important cause for early post-settlement mortality in corals. The corallivore could reinforce recruitment bottlenecks and reduce coral recovery after disturbances.

Introduction

Coral recruitment is essential to the regeneration of reefs following disturbances (Connell et al. 1997; Hughes and Tanner 2000). Reefs that have been severely damaged are reliant on the input of larvae from surviving corals and their subsequent settlement, survival and growth. When approaching the reef in search of a suitable settlement spot, coral larvae are the prey of planktivorous fishes and corals (Fabricius and Metzner 2004). Once settled, coral spat can be overgrown by other benthos, smothered by sediments, or preyed upon by corallivores (Ritson-Williams et al. 2009). Despite the recognized importance of early post-settlement mortality in shaping coral populations and influencing reef recovery (Connell et al. 1997; Hughes and Tanner 2000), studies addressing the causes of mortality early after settlement have been hampered by the small size and cryptic behavior of newly settled spat, which makes them difficult to find and observe *in situ* (Vermeij and Sandin 2008). Thus, most available evidence for these causes relies on repeated censuses through time (Vermeij and Sandin 2008; Penin et al. 2010) or indirect observations (Bak and Engel 1979; Box and Mumby 2007).

While coral predators, or corallivores, can cause mortality and injuries in adult corals (Rotjan and Lewis 2008), they are also a major cause of mortality in young corals (Brock 1979; Mumby 2009). Grazers help reduce algal overgrowth of corals and have a net positive effect on coral recruitment (Mumby 2009). However, they can incidentally remove coral recruits and strongly influence spatial patterns of early stage mortality of corals (Christiansen et al. 2009; Penin et al. 2010). Much less is known of predation by non-grazing corallivores, in particular invertebrates, on coral settlers, and direct observations of predation by these organisms are scarce. Yet, there are more than 50 invertebrate species known to consume live coral tissue (Rotjan and Lewis 2008). Furthermore, corallivores can shift in diet as they grow and change habitat (Zann et al. 1987). Reciprocally, predation risk in corals can differ from larval, post-settlement and adult stages (Fabricius and Metzner 2004; Christiansen et al. 2009; Ritson-Williams et al. 2009). Experimental evidence for

such ontogenic shifts in either the diet of corallivores or the susceptibility to predation for different developmental stages of corals is lacking.

The amphinomid fireworm *Hermodice carunculata* is a widespread and abundant corallivore in tropical and temperate seas. It is a well and long known predator of scleractinian corals (Marsden 1962; Ott and Lewis 1972) and a vector of the coral bleaching pathogen *Vibrio shiloi* (Sussman et al. 2003). In this study, we examine coral predation by *H. carunculata* when offered settlers (< 3-weeks-old) and adults of two species of Caribbean corals, *Montastraea faveolata* (broadcast spawner) and *Agaricia humilis* (brooder), under laboratory conditions.

Materials and methods

Field collections and coral settlement

Experiments were performed at the Carmabi research station in Curaçao, Southern Caribbean. All organisms used for experimentation were collected from reefs adjacent to the station. *M. faveolata* gametes were collected from 8 colonies which spawned on 17 October 2011 at the shallow fore-reef (3-8 m) at the Seaquarium (12°04'N, 68°53'W). Gamete collection, fertilization and rearing of the planulae were done according to Vermeij et al. (2009). 5-days-old larvae were placed in the presence of small fragments (3 cm²) of the CCA species *Paragoniolithon* sp. for 48h. Planulae displayed high settlement rates (> 80%) for all CCA fragments within 24h and metamorphosed within 12h. Experimental substrates with 10 coral spat each were prepared from each CCA fragment by haphazardly removing excess spat with a razor blade under a binocular microscope.

A. humilis larvae were released from 9 mature colonies collected from Marie Pompoen (12°05'N, 68°54'W). The larvae were released between 1 and 3 October 2011. They were left to settle on the plastic walls and bottom of polyethylene containers. Experimental substrates were prepared by cutting the containers into small plastic fragments harboring 10 spat each. *M. faveolata* and *A. humilis* settlers

were 2-days and 3-weeks-old respectively when used for experimentation. All were still at the primary polyp stage.

As *M. faveolata* and *A. humilis* typically form large (> 10 cm in diameter) and small (up to 4-6 cm diameter) colonies respectively, *M. faveolata* adult fragments consisted of small (2.5-5 cm diameter) patches of living tissue separated from a “mother” colony by fission to avoid tissue damage during collection, while *A. humilis* fragments were entire colonies (3-6 cm diameter). Samples were collected using a hammer and chisel at the shallow fore-reef (3-8 m) at Buoy Zero (12°07'N, 69°57'W) for *M. faveolata* and at Marie Pompoen for *A. humilis*. Fireworms were collected at the shallow fore-reef (3-8 m) at Buoy Zero. Adult coral fragments and worms were kept in 1-l containers with constant water flow-through to adapt to laboratory conditions for 5 days without being fed prior to experimentation.

Feeding experiments

Each experimental unit was made by placing a substrate with 10 coral spat, an adult coral fragment and a piece of terracotta to serve as shelter for the fireworms into a 1-l container. Containers were provided with constant flow-through of filtered seawater (100 µm mesh size) and were placed in an aquarium filled with enough seawater to serve as water bath. The aquarium was provided with flow-through seawater subject to natural daily temperature fluctuations and shaded to ensure light conditions similar to the depth range at which all organisms were collected (~ 30% of the incident surface photosynthetically active radiation).

Units were then randomly allocated to one of four predation treatments (n = 8 replicates each) with different numbers and size classes of fireworms to keep predator biomass approximately equal among treatments: (1) 8 small-sized (0.5-2.5 cm in length) fireworms added; (2) 4 mid-sized (2.5-5.5 cm) fireworms added; (3) 1 large-sized (5.5-7.5 cm) fireworm added; and (4) no fireworm added (control). The experiment was run for 4 days. Fireworms were observed daily between 15:30 and 17:30 h corresponding to their peak of activity in the field (Ott and Lewis 1972) and in the lab (A. Wolf, personal observation). Each time a worm fed on a spat, feeding

duration and worm size class were recorded. Coral spat and adult fragments were inspected each morning. Surviving spat were counted under a binocular microscope. Coral fragments were photographed and inspected for bite marks. At the end of the experiment, the correct taxonomy of all fireworms was verified using Fauchald (1977) and Yáñez-Rivera et al. (2009).

This experiment was run using *A. humilis* on 22–25 October 2011 and repeated using *M. faveolata* on 27–30 October 2011. Differences among treatments in spat survival rates for each species were analyzed using Kaplan-Meier log-rank survival analysis, followed by pairwise comparisons among treatments using the Holm-Sidak procedure. Although coral species were run in successive experiments, we pooled data of both experiments and used Cox proportional hazard regression analysis to have an indication of differences in survival rates between coral species. Differences in bite marks were analyzed using Kruskal-Wallis test.

Results and discussion

Spat survival significantly differed among predation treatments in both experiments and increased with fireworm size (Fig. 1; Table 1a). Coral settlers had the lowest probabilities to survive in the presence of small-sized fireworms (< 2% on Day 4 for both coral species). Survival probabilities increased to 42% for *M. faveolata* and 54% for *A. humilis* in the presence of mid-sized fireworms, followed by 93 and 90% in the presence of large-sized fireworms. Both controls had settler survival probabilities above 97%. Pairwise comparisons within each coral species indicated significant differences among all treatment combinations, except between the large-sized fireworm and control *M. faveolata* treatment (Table 1b). Large-sized fireworm treatments showed weak ($P = 0.016$ for *A. humilis*) to non-significant ($P = 0.148$ for *M. faveolata*) differences with the controls, unlike small- and mid-sized worm treatments ($P < 0.001$).

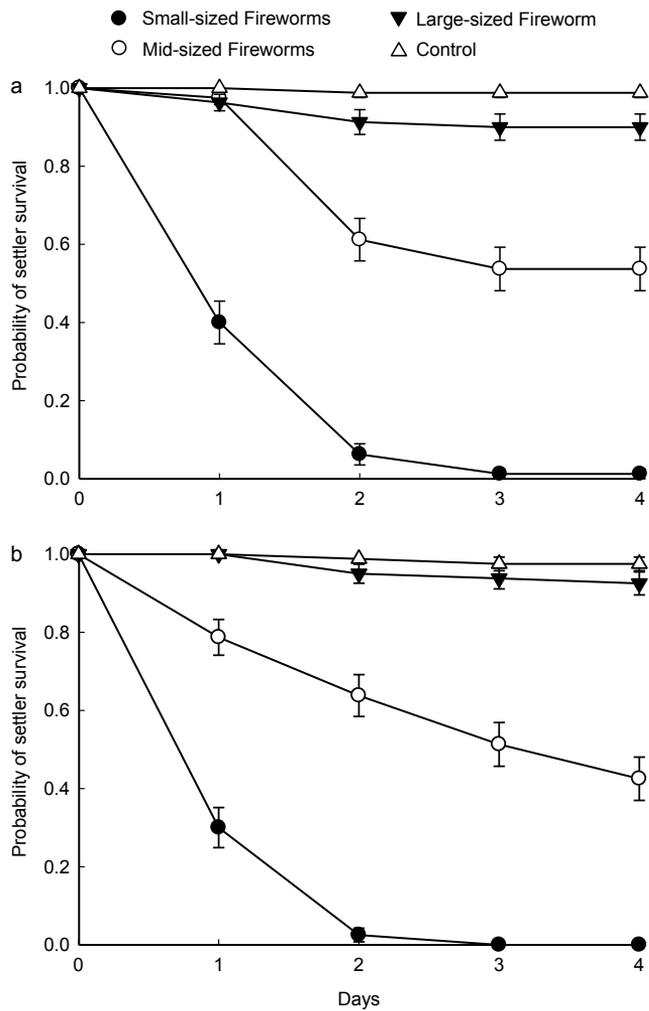


Fig. 1 Kaplan-Meier estimated probability of settler survival (mean \pm SE, n = 8 replicates) as a function of time in each treatment for **a)** *Montastraea faveolata* and **b)** *Agaricia humilis* settlers

Table 1 a) Median survival time of coral settlers in days (mean \pm SE, n = 8) for each treatment for each coral species estimated by Kaplan-Meier log rank Survival Analysis. **b)** Pairwise multiple comparisons between treatments (Holm-Sidak Method) for each coral species, derived from Cox proportional hazard main effect model.

a) Coral species	Treatment				Log rank test		
	Small-sized fireworm	Mid-sized fireworm	Large-sized fireworm	Control	Test-statistic	df	P
<i>Montastraea faveolata</i>	1.33 \pm 0.06	2.94 \pm 0.14	3.89 \pm 0.06	3.96 \pm 0.04	296.915	3	<.0001
<i>Agaricia humilis</i>	1.48 \pm 0.07	3.13 \pm 0.11	3.78 \pm 0.09	3.98 \pm ∞	278.275	3	<.0001

b) Treatment	<i>Montastraea faveolata</i>				<i>Agaricia humilis</i>			
	Small	Mid	Large	Control	Small	Mid	Large	Control
Small-sized fireworm	-				-			
Mid-sized fireworm	0.000	-			0.000	-		
Large-sized fireworm	0.000	0.000	-		0.000	0.000	-	
Control	0.000	0.000	0.148	-	0.000	0.000	0.016	-

Cox proportional hazard regression analysis indicated no differences in survival rates between experiments (Cox hazard ratio = 1.16; 95% CI = 0.85, 1.57). There was also no difference between a Cox saturated model (that included experiment-treatment interactions) and a Cox main effects model (without interactions) (chi-square test: $\chi^2 = 1.43$, $df = 3$, $P = 0.69$), suggesting that the effects of *H. carunculata* on spat survival did not differ between coral species.

Fireworms hardly predated on adult coral fragments. Bite marks were found on two *M. faveolata* colonies and none on *A. humilis* colonies. No differences in the number of bite marks per fragment were detected among predation treatments for *M. faveolata* (Kruskal-Wallis test, $H = 6.09$, $df = 3$, $P = 0.1$). Fragment size of adult samples did not differ between treatments (1-way ANOVA on log transformed data, $P > 0.05$ for both coral species) and thus could not have affected these results.

Feeding observations validated our results. Predation was only observed on coral settlers for small- and mid-sized fireworms (Fig. 2).



Fig. 2 Time series photographs of predation on a 3-months-old *Favia fragrum* spat by *Hermodice carunculata* **(a)** at start of the experiment, 2 mid-sized worms are placed in a 10 cm diameter petri dish filled with 50 mL of filtered seawater, harboring two coral recruits, **(b)** at Day 2 when one worm detects a coral spat, the enlargement of the buccal mass, a muscular area comprising the pharynx and oesophagus, is visible, **(c)** 2 minutes after spat detection, the buccal mass is fully everted over the coral spat, rippling muscles and secreted enzymes break down the coral tissue and ingest it, and **(d)** 4 minutes after spat detection, the fireworm stops feeding and leaves its prey; more than half of the coral tissue was eaten. Scale bar (identical for all photographs) = 5 mm.

When small-sized fireworms encountered a spat, they immediately proceeded with feeding. In contrast, large-sized fireworms generally passed by without slowing down in speed, while mid-sized worms had an intermediate behavior. Average feeding duration decreased with fireworm size, lasting 9.2 ± 0.5 and 4.5 ± 0.3 minutes ($n = 11$ and 6 observations) for small- and mid-sized worms respectively for *M. faveolata* and 9.7 ± 0.7 and 4.0 ± 0.6 minutes ($n = 9$ and 3) respectively for *A. humilis* settlers. These feeding times are within the lower range of previous observations (e.g. 2-88 mins in Witman 1988). Generally, encounters with coral settlers appeared fortuitous since fireworms would frequently pass nearby without changing direction, supporting the stronger importance of gustation over olfaction during the foraging process of amphinomids (Lindsay 2009).

Ontogenic shifts in diet and predation risk are ubiquitous in many size-structured organisms (Werner and Gilliam 1984). Our results support an ontogenic shift in the diet of *H. carunculata* and a change in coral susceptibility to predation by *H. carunculata* between settlers and adults. The causes of these changes remain to be investigated. A number of studies on marine organisms suggest that the selection of small preys may not be related to foraging experience or energy gain, but represent an adaptation to choose preys in habitats protected from predation (Mittelbach 1981; Gosselin and Chia 1996; Gosselin 1997; Schoepf et al. 2010). Large-sized fireworms are commonly less cryptic than small-sized individuals (A. Wolf, personal observations). It remains to be investigated whether such behavior is the result of predation, but it is possible that a reduction in predation risk with fireworm size triggers a suite of ontogenic changes, including a shift from complex to more open habitat and a search for larger preys.

This experiment was conducted in laboratory settings lacking some ecologically realistic conditions (e.g. restricted prey choice and settlement substrata) and using only two coral species. Therefore, the applicability of this study in the field remains to be investigated. However, our study demonstrates that *H. carunculata*, in particular small-sized individuals, feed on coral settlers and thus

should be considered as a potential cause for early post-settlement mortality in corals. Several coral species, in particular long-lived corals such as *Montastraea*, appear to have limited potential for recovery due to bottlenecks in recruitment (Edmunds 2002; Mumby 2006). Such predation could affect the persistence of these species.

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Appendix



Hermodice carunculata Close-up photograph taken at the ship wreck Superior Producer at 30 m water depth. This specimen (+ 15 cm body length) was one of 10 or more individuals of similar size, all feeding on scleractinian corals of the genus *Tubastrea* (*Tubastrea coccinea*). Agglomerations of fireworms that size were never observed on reef crests.

5

Synergistic effects of algal overgrowth and corallivory on reef-building corals

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Abstract

Humans impact ecosystems in a multitude of ways, yet the interactive effect of multiple stressors on ecological communities is poorly known. Algal competition and corallivory are two major stressors contributing to the decline of coral reefs. Here, we provide the first evidence of algal-induced corallivory and synergistic effects between the two stressors on corals. When corals (*Montastraea faveolata*) were placed in contact with algae (*Halimeda opuntia*) together with corallivorous fireworms (*Hermodice carunculata*) in aquaria, corals suffered high tissue mortality. This mortality was reduced in the presence of algae only, and no mortality occurred in the presence of fireworms only or when excluding both algae and fireworms. These findings were supported by field observations showing a predominance of fireworms inside algae contacting live corals, and by an in situ experiment demonstrating higher coral mortality in contact with algae left undisturbed than with algae from which all mobile epifauna were periodically removed. Our results suggest that reefs could become more prone to corallivory under high algal abundance. This study highlights the potential complex interactions that govern the relationships between corallivores, algae and corals on present-day reefs.

Introduction

Across the globe, human activities increase the number and severity of disturbances impacting natural systems. Multiple stressors combine in unexpected ways to cause drastic changes in the relative abundances of major species groups within the wider community, resulting in significant losses of habitat, biodiversity and ecosystem function (Bulling et al. 2010). A topical example of such ecosystem transformation is the “phase shift” of coral reefs from coral to algal dominance (Hughes 1994; Bruno et al. 2009). A consensus has emerged that these shifts are initiated by sudden events, such as bleaching, diseases or storms, which push reefs beyond their stable coral-dominated state, rather than by the increase in benthic algae *per se* (Aronson and Pretch 2006). However, as benthic algae overtake newly available substrate following coral mortality events, algae increase in abundance and become major competitors with corals (Hughes et al. 2007). Algal competition may be affected by a number of environmental and anthropogenic disturbances, such as eutrophication (Smith et al. 1981), ocean acidification (Diaz-Pulido et al. 2011) and loss of herbivore grazing (Hughes et al. 2007). Potential interactions between algal competition and corallivory have not been addressed.

Corallivory is an important, but often underestimated, biotic stressor for coral reefs (Rotjan and Lewis 2008). In the Indo-Pacific, the seastar *Acanthaster planci* kill more coral than any other disturbances (Fabricius et al. 2010). In the Caribbean, corallivores have not been thought of as constituting a significant cause of coral mortality (Mumby 2009). However, a number of invertebrate corallivores have recently been found to be associated with coral diseases or to act as vectors of coral pathogens, such as the amphinomid fireworm *Hermodice carunculata* (Sussman et al. 2003; Miller and Williams 2006) and the corallivorous snail *Coralliophila abbreviata* (Williams and Miller 2005). Corallivorous invertebrates are common algal dwellers, presumably for food, shelter and/or recruitment. These organisms could influence coral-algal interactions through feeding and transmission of disease (Mumby 2009).

We investigated the role of the corallivorous fireworm *H. carunculata* in interactions between the green calcareous alga *Halimeda opuntia* and Caribbean reef-building corals. *H. opuntia* provides a complex 3-dimensional framework known to host a wide variety of invertebrates, including fireworms (Naim 1988). It is also known to cause coral mortality and disease (Nugues et al. 2004). *H. carunculata* is commonly observed feeding on diseased corals (Miller and Williams 2006) and has been shown to act as a vector of the coral-bleaching pathogen *Vibrio shiloi* in the Mediterranean (Sussman et al. 2003). We hypothesized that corallivory by the worm could enhance mortality in corals interacting with the alga. First, we investigated the abundance of the worm in algae overgrowing different coral species and related its abundance with coral health in the field. Second, we tested the effects of whole algal-associated epifauna on coral bleaching, death and disease in interactions between *H. opuntia* and the coral *Montastraea faveolata* in a field experiment. Finally, we examined the separate and combined effects of the algae and the worm on coral bleaching and mortality under laboratory conditions. This last experiment was also performed using another corallivore, the snail *C. abbreviata*.

Material & Methods

Field survey

If *Hermodice carunculata* is using *Halimeda opuntia* to feed on corals, it should be more abundant in algae overgrowing living corals compared to algae exclusively covering dead coral skeleton, and its abundance should be negatively associated with coral health. Additionally, since corallivores commonly exhibit prey preferences (Rotjan and Lewis 2008), their abundance in the algae could differ among coral species. To test these hypotheses, clumps of *H. opuntia* overgrowing live tissue from 5 coral species (*M. faveolata*, *M. cavernosa*, *Siderastrea siderea*, *Colpophyllia natans*, *Diploria strigosa*) and dead coral skeleton only (n = 11 - 14 replicates) were haphazardly collected using ziplock bags by swimming along the reef terrace (4 – 8 m water depth) at Carmabi Buoy One (12°07.551'N,

68°58.529'W) in Curaçao, Southern Caribbean. Clumps overgrowing dead coral skeleton only were collected > 1 m away from any living corals. We also collected *H. opuntia* overgrowing the coral *M. faveolata* and algae overgrowing dead coral skeleton only, at three other sites on the island (Marie Pompoen 12°05.525'N 68°54.516'W, Spanish Water 12°03.790'N 68°51.190'W and Playa Kalki 12°22.530'N 69°09.470'W). After removing each clump of algae overgrowing live coral tissue, coral health underneath the algae was scored as follows: 1, tissue normal; 2, tissue partially bleached; 3, tissue completely bleached; 4, tissue < 50 % dead; 5, tissue ≥ 50 % dead. Back in the laboratory, all fireworms were separated from the algal segments, identified microscopically following Fauchald (1977), and counted.

Note that the goal of this study was not to characterize all potentially corallivorous species present in the samples. *H. carunculata* was the largest corallivore commonly found and thus likely involved in coral tissue damage. The corallivorous snail *C. abbreviata* was also present, but remained rare. Only 5 of 146 collected *H. opuntia* clumps contained *C. abbreviata*. In contrast, 71 clumps contained *H. carunculata*. The negligible impact of *C. abbreviata* associated with *H. opuntia* was supported by the lack of relationship between the snail abundance in the clumps and tissue condition under the alga (data not shown), as well as the result of our laboratory experiment (see results). Thus, data on *C. abbreviata* from the field survey were not presented. Sampling was carried out between March and April 2010.

Field experiment

We tested the effect of *H. opuntia*-associated epifauna on corals at Carmabi Buoy Zero (12°07.417'N, 68°58.592'W) in Curaçao. Colonies of *Montastraea faveolata* were haphazardly chosen swimming along the reef terrace. They were used in the experiment if the colonies showed no sign of disease and were not overgrown by algae. The coral *M. faveolata* was chosen for this study as it is a major reef building species, and adult corals are abundant. Also, our field survey had indicated a high

abundance of fireworms in *H. opuntia* in contact with this species. The colonies were tagged and randomly allocated to 1 of the following 5 treatments (n = 20 colonies each): (T1) *H. opuntia* added: a clump (~ 90 g wet weight) of *H. opuntia* with all epifauna removed in the lab was put inside a nylon mesh bag (1 cm mesh) and positioned to overgrow ~ 90 cm² of living coral surface using rubber bands and nails inserted onto nearby dead coral substrate, with no further manipulation; (T2) *H. opuntia* added, epifauna removed: as with T1 but the mesh bag was retrieved every 5 days, all epifauna was removed from the alga in the lab, and the mesh bag with the alga was returned to its initial position on the same day; (T3) *H. opuntia* mimic: a plastic aquarium plant of similar structure and size was used instead of the living alga, the epifauna was not removed during the experiment; (T4) procedural control: only a nylon mesh bag was placed in contact with the coral tissue; (T5) control: the coral colony was left untouched. The *H. opuntia* mimic treatment was used to distinguish between the biological and physical properties of the alga. In addition, 20 nylon mesh bags with similarly sized clumps of *H. opuntia* were placed onto dead coral substrate > 1 m away from any living corals (T6) to control for the effect of the coral contact with the alga on *H. carunculata*.

The experiment was run for 25 days (20 March – 14 April 2010). All replicates were checked every 5 days. *H. opuntia* remained healthy for the duration of the study. At the end of the experiment, the mesh bags were collected using ziplock bags. The surface areas of recently dead coral, bleached and healthy tissue underneath the mesh bag were measured to the nearest cm² using a flexible transparent plastic grid divided into 100 1 cm x 1 cm square cells and were expressed as percentage of total area covered by the bag. The controls (T5) suffered no mortality and had 100 % healthy tissue. In addition, signs of white plague, in the form of recently dead tissue characterized by a sharp and regular demarcation between apparently healthy coral tissue and freshly denuded skeleton often extending beyond the algal overgrown area were recorded. Back in the laboratory, all epifauna were removed from the living or mimic *H. opuntia* and mesh bags. *H. carunculata* were identified and counted.

Laboratory experiment

The field experiment had its limitations since we could not remove *H. carunculata* without removing the remainder of the epifauna, access by *H. carunculata* could not be controlled and the algae had to be periodically disturbed to remove the epifauna. We therefore performed a laboratory experiment in which we could control the presence/absence of *H. carunculata* without disturbing the algae and study the independent and combined effects of the fireworm and *H. opuntia* on *M. faveolata*. Four outdoor 1 m³ (1 m x 1 m x 1 m) aquaria were each split in two equal sections, provided with flow-through seawater (~4-5 l min⁻¹) subject to natural daily temperature fluctuations (range 29.4 to 30.8 °C) and shaded to ensure light conditions similar to the depth range at which all organisms were collected (~ 30 % of the incident surface photosynthetically active radiation).

One section of each aquarium was randomly allocated to one of two corallivore treatments: (i) corallivores present: 30 fireworms (ca. 3 - 6 cm in length, collected in *H. opuntia* clumps) were added, and (ii) corallivores absent: no fireworms added. Within each section, 6 coral fragments of similar size (~ 300 cm²) and free of epifauna were placed and randomly allocated to one of two algae treatments: (i) *H. opuntia* present: a clump (~ 90 g wet weight) of *H. opuntia* with all epifauna removed was placed inside a nylon mesh bag (1 cm mesh) and positioned to overgrow ~ 90 cm² of living coral surface with cable ties, and (ii) *H. opuntia* absent: no *H. opuntia* and mesh bag were added. Coral fragments were 20 to 25 cm apart. All organisms were collected on the reef terrace at Carmabi Buoy Zero. Coral fragments were removed from the reef by breaking off columns below the live coral to avoid tissue damage and acclimated for 5 days in the experimental aquaria prior to experimentation. The same experiment was also carried out using the corallivorous snail *C. abbreviata* and the coral *Montastraea annularis* which is commonly infested by the snail (Baums et al. 2003). In this second experiment, 60 snails were added to each section assigned to the corallivores present treatment.

The experiments were run for 24 days (16 October to 9 November 2010 for *H. carunculata* and 18 November to 12 December 2010 for *C. abbreviata*). Tank

surfaces were cleaned every 2 days with scouring pads. All replicates were checked during the tank cleaning process. *H. opuntia* and corallivores remained healthy for the duration of the study. At the end of the experiment, the mesh bags were collected using ziplock bags and the surface areas of recently dead coral, bleached and healthy tissue were measured as described above. Complete fragment mortality was also recorded. Upon complete death, fragments showed rapid tissue disintegration and white plague signs typical of the natural conditions were not observed.

Statistical analyses

Data from our field survey and field experiment violated parametric assumptions, so non-parametric statistics were used. Differences among contact substrates (different coral species and dead coral) in the field survey and treatments in the field experiment were assessed using a Kruskal-Wallis ANOVA on Ranks, followed by Student-Newman-Keuls posthoc tests on Ranks (Zar 2010). A Scheirer-Ray-Hare test, a non-parametric equivalent of a 2-way ANOVA, was used to evaluate effects of overgrown substrata (*M. faveolata* and dead coral) and sites on fireworm abundance. Differences in coral health scores were tested using an ordinal logistic regression including fireworm abundance in *H. opuntia* as covariate. To determine whether the worms were aggregated, the frequency distribution of the number of worms per algal clump with 0 to 4 or more worms was tested for goodness of fit to a Poisson distribution with a G-test after Williams's correction to avoid type I error (Sokal & Rohlf 1995). For this test, sites and coral species were pooled. However, separate analyses were run for algal clumps contacting live and dead corals (n = 95 and 51 respectively) to determine the influence of live coral on the worm behavior. Differences in the prevalence of white plague were analyzed using a heterogeneity G-test, followed by a simultaneous test procedure to determine homogeneous groups (Sokal & Rohlf 1995). Data for the aquarium experiment were arcsine square root transformed to conform to assumptions of normality and homogeneity of variance and analyzed by a factorial 3-way ANOVA with plot (i.e. aquaria) as random factor and corallivores and algae treatments as fixed factors.

Results

Field survey

All the fireworms found in *H. opuntia* were identified as being *H. carunculata*. At Carmabi Buoy One, their abundance in the algal clumps varied significantly among overgrown substrata (Kruskal-Wallis ANOVA on ranks, $H = 16.20$, $df = 5$, $P = 0.006$) (Fig. 1a).

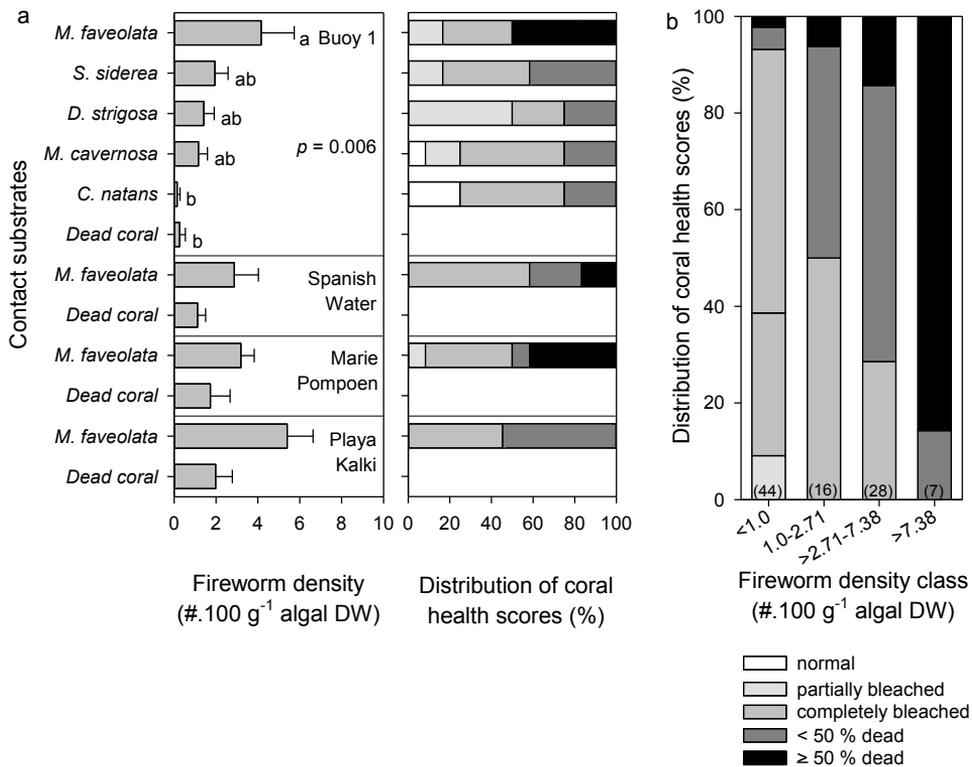


Fig. 1 (a) Fireworm abundance in *Halimeda opuntia* (means \pm SE, $n = 11 - 14$) and scores of coral health for all 6 contact substrata at Carmabi Buoy One and for *Montastraea faveolata* and dead coral skeleton at 3 other sites on Curaçao. Data on fireworm abundance at Carmabi Buoy One were analyzed by Kruskal-Wallis ANOVA on Ranks. Letters indicate homogeneous subgroups by posthoc Student-Newman-Keuls tests. **(b)** Scores of coral health observed underneath *Halimeda opuntia* as a function of fireworm density (logarithmic scale). Number of interactions for each density class is in parenthesis. All coral species and sites were pooled. DW = Dry weight.

There were more fireworms in *H. opuntia* overgrowing *M. faveolata* than in algae overgrowing dead coral or *C. natans*, but not the three other coral species. The difference in fireworm abundance between *M. faveolata* and dead coral was consistent among sites, with site having no influence (Scheirer-Ray-Hare test, $P < 0.001$ for overgrown substratum, $P = 0.13$ for site and interaction). Negative relationships between fireworm abundance and tissue condition under the algae were found for *M. faveolata* and *S. siderea* at Carmabi Buoy One and for *M. faveolata* at Spanish Water (intercepts = 0.73 ± 0.33 , 0.87 ± 0.41 and 0.44 ± 0.22 , $P = 0.028$, $P = 0.035$ and $P = 0.042$, respectively). Pooling species and sites, the negative effect of fireworm abundance on coral health was highly significant (intercept = 0.43 ± 0.08 , $P < 0.001$, Fig. 1b). The frequency distribution of worms in the algal clumps overgrowing both live and dead corals significantly differed from a Poisson distribution (G -test, $G_{\text{adj}} = 48.83$ and 27.48 respectively, $df = 4$, $P < 0.001$), demonstrating aggregative behavior in both cases. However, the G_{adj} value was markedly higher for live compared to dead coral, suggesting that this behavior is enhanced by the presence of live coral.

Field experiment

The number of fireworms removed every 5 days in the *H. opuntia* added, epifauna removed (T2) treatment, averaged $1.09 (\pm 0.26 \text{ SE})$ individuals per 100 g algal dry weight ($n = 100$) and did not differ among retrieval times (Kruskal-Wallis ANOVA on ranks, $H = 3.65$, $df = 4$, $P = 0.45$). At the end of the experiment, there were significantly more fireworms in the *H. opuntia* added (T1) treatment compared to all other treatments, including the epifauna removed (T2) treatment (Fig. 2a), indicating that the removal treatment effectively reduced the abundance of fireworms. Paralleling patterns of fireworm abundance, the percentage of recently dead tissue was significantly higher in the *H. opuntia* added (T1) treatment compared to all other treatments, including the epifauna removed (T2) and *H. opuntia* mimic (T3) treatments (Fig. 2b).

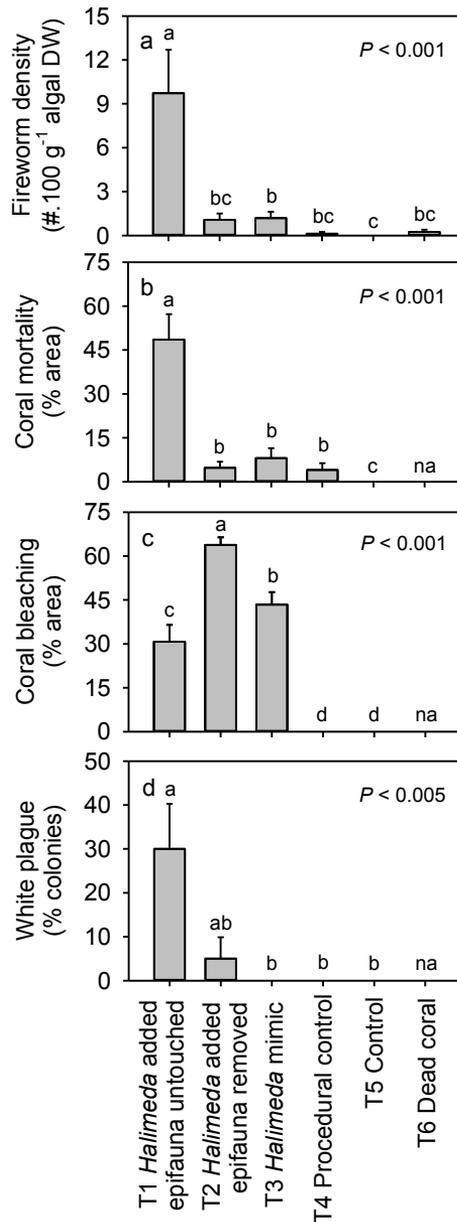


Fig. 2. Effect of algal-associated epifauna on *Montastraea faveolata*. **(a)** Fireworm abundance in mesh bags or experimental control area, **(b)** coral mortality, **(c)** coral bleaching, and **(d)** prevalence of white plague for the different treatments. Analyzed by Kruskal-Wallis ANOVA on Ranks (a-c) or heterogeneity G-test (d). Letters indicate homogeneous subgroups by posthoc Student-Newman-Keuls tests (a-c) or simultaneous test procedure (d). na = not applicable. Values are means \pm SE (n = 20 colonies for T1 to T5 or bags for T6).

These results suggest that the epifauna increased coral mortality in contact with living algae, but that this effect was not reproduced using mimic algae. The percentage of bleached tissue was highest in the epifauna removed (T2) treatment, followed by the mimic (T3) and *H. opuntia* added (T1) treatments (Fig. 2c). Thus, coral bleaching did not increase in response to the epifauna and seemed largely caused by physical mechanisms.

Signs of white plague were observed in 6 (30%) of the 20 transplants in the *H. opuntia* added (T1) treatment, only 1 (5%) of the transplants in the epifauna removed (T2) treatment and in none of the transplants in the other treatments. Differences among treatments were significant (heterogeneity G-test, $G_H = 39.58$, $df = 4$, $P < 0.005$, $n = 20$) (Fig. 2d). STP tests indicated that {T1, T2} and {T2, T3, T4, T5} formed two homogenous groups. Thus, these results support a positive trend between the algae and coral disease, but we cannot conclusively attribute the disease to the sole effect of the algal-associated epifauna.

Laboratory experiment

In the experiment with *H. carunculata*, coral mortality was affected by both *H. opuntia* and the presence of the predator, as indicated by the significant interaction between these factors (Fig. 3a). In the absence of algae, coral mortality was not recorded regardless of the predator presence or absence. However, when corals were overgrown by algae, the presence of *H. carunculata* increased coral mortality (2-way ANOVA with plot and corallivores, $F_{1,19} = 4.97$, $P = 0.038$).

This effect did not occur with *C. abbreviata* (Fig. 3b). Coral bleaching was only affected by *H. opuntia*, with significantly increased bleaching with the algae present (Fig. 3c, d). Both corallivores had no influence on coral bleaching. Only 1 and 2 fragments in the experiments with *H. carunculata* and *C. abbreviata* respectively died, so no significant trends were detected in the occurrence of whole fragment mortality.

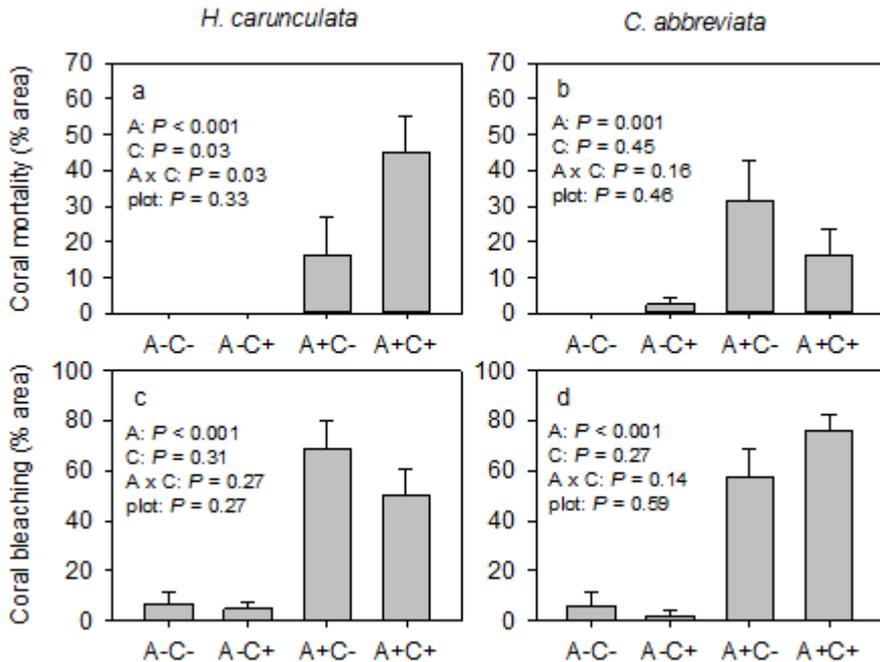


Fig. 3. Independent and combined effects of *Halimeda opuntia* and corallivores on coral mortality (a, b) and bleaching (c, d). Data are for *Hermodice carunculata* on *Montastraea faveolata* (a, c) and *Coralliophila abbreviata* on *Montastraea annularis* (b, d). C = corallivores treatment and A = algae (*Halimeda opuntia*) treatment. Statistics are from a factorial 3-way ANOVA. Values are means \pm SE (n=4).

Discussion

The results of this study provide a robust demonstration of synergistic effects between predation by one species of invertebrate corallivore and overgrowth by one algal species on coral mortality. Contact with algae together with the addition of fireworms produced high coral tissue mortality. This mortality was significantly reduced in the presence of algae only, and no mortality occurred in the presence of fireworms only or in the control with no algae or fireworms. Both hypotheses regarding the distribution of *H. carunculata* in *H. opuntia* and their association with coral health in the field were validated, lending further support for the relevance of

our experimental results. Fireworm abundance was higher in algae overgrowing the coral *M. faveolata* than in algae growing exclusively on dead coral, and increased fireworm abundance was associated with decreased coral health in the form of coral bleaching and mortality in the contact zone between corals and algae. Finally, the field experiment showed increased coral mortality in contact with *H. opuntia* in the presence of epifauna compared to the algae with epifauna removed. It cannot be excluded that epifauna other than *H. carunculata* contributed to this mortality. However, given the predominance of the worm in *H. opuntia*, its association with coral health and the results of our laboratory experiment, *H. carunculata* is likely to have been a major contributor.

In the field experiment, mimic algae did not produce the high coral mortality observed with living algae. There are two possible explanations of this result. First, the algal mimic may not be the preferred habitat for *H. carunculata* and reduced worm densities in algae should, in principle, lower their effects on overgrown corals. While this explanation is plausible, fireworms were found in the mimic algae and we observed them in a wide variety of other refuges, such as crevices, marine debris and other macroalgae. Alternatively, these results imply that algal-induced corallivory may be associated with chemical instead of physical effects produced by the algae. Although benthic algae can affect corals via a number of physical mechanisms (e.g. abrasion, shading) (McCook et al. 2001), coral mortality caused by algae, including *H. opuntia*, often involves the release of algal exudates (Smith et al. 2006; Rasher and Hay 2010). The most likely mechanism is that *H. carunculata* was initially attracted by dying tissues produced by algal-induced chemical effects and thereafter both stressors acted in synergy to cause further coral mortality.

There is a precedent for corallivores to prefer damaged coral tissue as food source. For instance, the coral-feeding wrasse *Labrichthys unilineatus* has been shown to exhibit a strong preference for damaged coral tissue (McIlwain and Jones 1997). On Lizard Island, several species of corallivorous fishes selectively targeted disease lesions over adjacent healthy coral tissues (Chong-Seng et al. 2011). Such preferences may be caused by a release of olfactory attractants, a reduction in

feeding deterrents (e.g. nematocysts) and/or higher energy rewards in damaged tissue (McIlwain and Jones 1997). *H. carunculata* possesses well-developed olfactory organs which could facilitate prey detection (Lizama and Blanket 1975). Since the algal mimics attracted a low abundance of worms although inducing high coral bleaching, the presence of dying tissue as opposed to bleached tissue may be essential to this detection, and metabolites leaking out dying tissues could act as olfactory attractants.

Once the corallivores are attracted to the zone of interaction, the mechanisms leading to coral mortality remain to be elucidated. Gut contents of *H. carunculata* have been found to include coral tissue (Marsden 1963), confirming that the worm can feed on corals. Aggregative feeding behavior of predators, in particular scavengers, can cause high mortality in preys subject to even a small degree of stress and/or damage (Ramsey and Kaiser 1998). In our study, the worms showed a marked aggregation behavior in *H. opuntia*, which appeared to be further enhanced by the presence of live coral. The alga is sufficient to damage and weaken corals, as confirmed by our algal treatments without corallivores. It may thus lead to high coral mortality by enhancing the aggregative behavior of *H. carunculata*. The worms may also modify the physical, chemical and microbial microenvironment of overgrown corals, such as by excreting particulate wastes and leaving remains of decaying tissues which can then be colonized by microbes, increase oxygen demand and reduce pH. These effects are likely to be exacerbated by the conditions of reduced flow underneath the algal canopy and to add on to the already detrimental effects of algae. Moreover, a number of corallivores, including *H. carunculata*, have been implicated as potential disease vectors (Sussman et al. 2003; Williams and Miller 2005). Our field experiment supported a relationship between *H. opuntia* and white plague signs, reinforcing earlier work at the same site (Nugues et al. 2004). However, although trends were suggestive of a role of the algal-associated epifauna in the disease process, differences in disease prevalence between algal treatments with and without epifauna were not significant. Corallivores may also reduce coral

disease progression through selective consumption of diseased coral tissues (Chong-Seng et al. 2011). Thus, their role in coral diseases is still equivocal.

The snail *C. abbreviata* was found in very low abundance in natural clumps of *H. opuntia* and our laboratory experiment provided no evidence of interaction between algal competition and corallivory by this species. *C. abbreviata* appears to be an obligate corallivore eating exclusively live coral (Ward 1965). In contrast, *H. carunculata* has been observed feeding on a variety of sessile organisms (Fauchald and Jumars 1979) and analyses of its gut contents indicated remains of corals, mollusks, polychaetes and crinoids (Marsden 1963). Therefore, it appears as a facultative coral feeder which nourishes on all kinds of food sources. Such feeding mode may allow the worm to opportunistically switch feeding towards corals damaged by algae. To date, there is no evidence for an increase in corallivores in the Caribbean. However, Caribbean reefs have experienced a drop in the cover of living coral and a rise in abundance of macroalgae (Hughes 1994; Bruno et al. 2009), so that corals more often face encroaching algae in their vicinity. The ensuing higher frequency of coral-algal encounters may increase food supply for facultative corallivores such as *H. carunculata*.

Although our field survey failed to detect significant differences among coral species in their health under the algae, algal-induced corallivory is likely to be species specific and to depend on the same factors influencing coral-algal interactions (McCook et al. 2001) and corallivory (Rotjan and Lewis 2008). The high concentration of worms on *M. faveolata* may be the result of a high feeding selectivity by *H. carunculata* and/or a high susceptibility to overgrowth by *H. opuntia*. Corallivores commonly exhibit preferences based on the nutritional status of the food, morphological characteristics (e.g. shape of the corallite, distance between polyps), or biochemical defense (McIlwain and Jones 1997). Furthermore, coral species exhibit different susceptibilities to different algae (McCook et al. 2001). A higher susceptibility to *H. opuntia* may result in a greater proportion of damaged tissue and attract more corallivores.

Evaluating the impact of corallivory on coral reefs has been equivocal because many corallivores appear to cause sporadic damage to corals (Mumby 2009). We investigated one species of algae and acknowledge that the limited scope of our study prevents broad generalizations on the interactive effects between algal competition and corallivory. However, numerous algal species harm corals (Rasher and Hay 2010) and thus could produce similar synergism. Interactive effects were not found for one (*C. abbreviata*) of the two experimentally studied corallivores. However, *H. carunculata* is distributed worldwide in tropical and warm temperate seas. Corallivores, in particular invertebrates which can easily find refuge in algae, form a highly diverse group (Rotjan and Lewis 2008). Invertebrates are often opportunistic feeders that due to their short life-spans and large diet can cope with rapidly changing environments. To date, there is evidence that they can interact with acute disturbances such as hurricanes to compromise recovery of some coral species (Knowlton et al. 1990). Their interactions with chronic disturbances such as algal competition could be easily unnoticed due to their small size, cryptic habits and little apparent damage and should be of concern in the face of accelerating and more severe disturbances on present-day reefs. Further research on corallivorous invertebrates, including their population size, their roles as disease vectors, their driving factors and possible remediation measures, may also prove useful in better managing reef resilience.

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Appendix A Photographs of the coral colonies from the different field experimental treatments

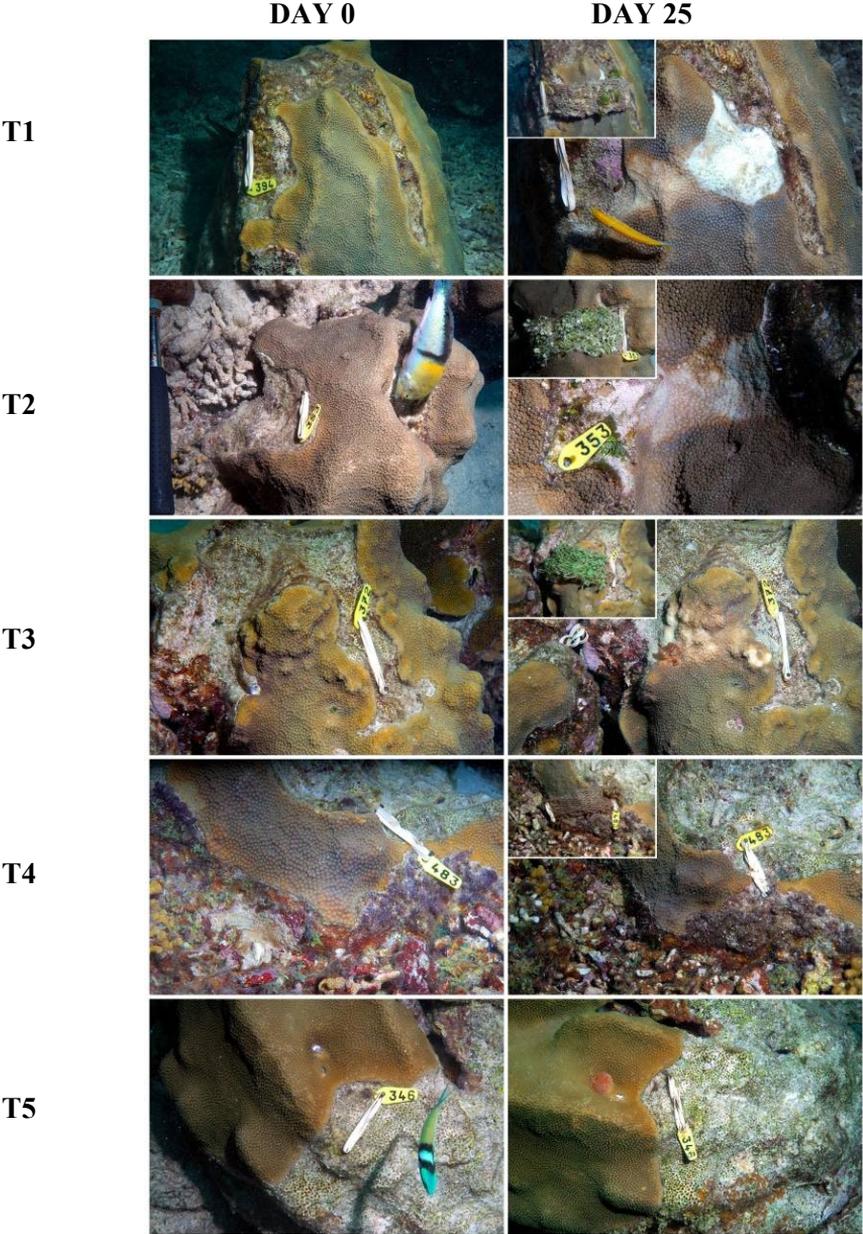


Fig A1. In situ time series photographs of the coral colonies from the different treatments at the start (Day 0) and end (Day 25) of the field experiment. *Insets* show the mesh bags with or without *Halimeda opuntia* covering the colonies after 25 days. The yellow tags are 46mm in length.

Appendix A Photographs of the coral colonies from the different laboratory experimental treatments

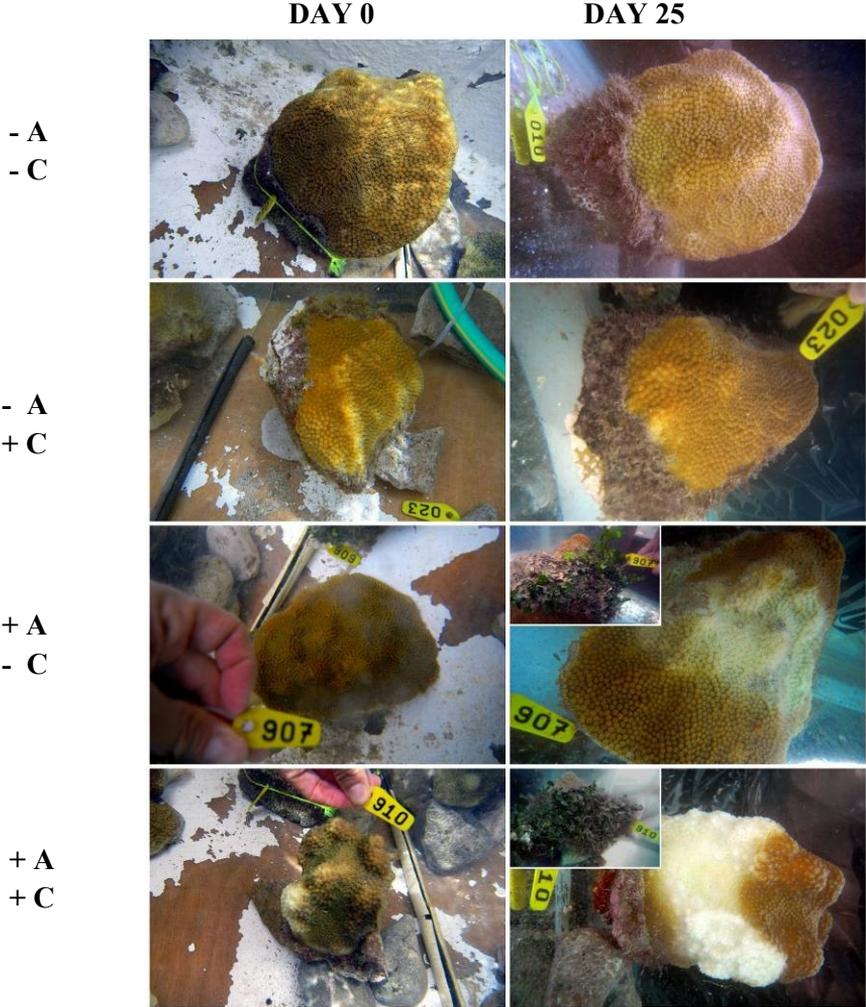


Figure A2. In situ time series photographs of the coral fragments from the different treatment combinations at the start (Day 0) and end (Day 25) of the laboratory experiment with *Hermodice carunculata*. Insets show the mesh bags with *Halimeda opuntia* covering the colonies after 25 days. C = corallivores (*H. carunculata*) treatment and A = algae (*H. opuntia*) treatment. The yellow tags are 46mm in length.

Synopsis

The resilience of a coral reef can be described as its capacity to absorb recurrent disturbances and adapt to change without fundamentally switching to an alternative stable state (Diaz-Pulido et al. 2009; Hoegh-Guldberg et al. 2007; Hughes et al. 2005; Scheffer and Carpenter 2003). This thesis has highlighted the role of macroalgae and corallivory on coral reef resilience in the Caribbean. Additionally, it furthered the understanding of these stressors and how they may influence resilience on a global scale (see Aronson and Precht 2006; Bruno and Selig 2007; Gardner et al. 2003; Hughes 1994). In particular, our findings illustrate 1) the effect of invertebrate-related corallivory under apparently natural, non-outbreak conditions, 2) elaborate further on direct coral-macroalgal interactions, and 3) reveal the existence of synergies between important stressors.

Evaluating the impact of corallivory on coral reefs has been equivocal since many corallivores cause sporadic damage to corals or have been assessed under population outbreak-related conditions (Colgan 1987; Dulvy et al. 2004; Knowlton et al. 1990; Mumby 2009). We assessed *Hermodice carunculata* occurrences under assumedly natural, non-outbreak conditions (Chapter 1) and estimate its impact to pose a permanent threat to coral spat, recruitment and reef replenishment (Chapter 4). Although such directed corallivory is unprecedented in literature, impaired growth and survival of juvenile scleractinian corals have been observed to occur due to vermetid gastropods (Lenihan et al. 2011). Herbivores also have the ability to impair coral recruitment due to accidental spat removal during foraging (Christiansen et al. 2009; Penin et al. 2010). However, grazing also enhances coral recruitment by preventing the establishment of turf and fleshy macroalgae and increasing settlement space for coral planulae (Burkepile and Hay 2010; Mumby 2009). Compared to *H. carunculata*, herbivores thus exert an overall positive effect on coral recruitment and coral reef resilience. Long-lived coral species such as *Montastraea* spp. appear to have limited potential for recovery due to bottlenecks in

recruitment (Edmunds et al. 2004; Mumby et al. 2006) and distinct predation by *H. carunculata* could affect the persistence of these species in the Caribbean.

We found no evidence for corallivorous behavior of *H. carunculata* on adult and healthy corals (Chapter 1, 4, and 5), which clearly separates this corallivore from others, such as the crown-of-thorns starfish *Acanthaster planci* (Pratchett 2007; Pratchett et al. 2009) and the gastropods *Drupella* and *Coralliophila* sp. (Brawley and Adey 1982; Cumming 1999; Turner 1994). Most studies on corallivory by *H. carunculata* are relatively dated (Marsden 1962, 1963a, 1963b; Ott and Lewis 1972), and consequences for coral reef functioning were estimated to be limited due to low population abundances of *H. carunculata* (Ott and Lewis 1972). Our findings indeed suggest that this invertebrate does not represent an immediate threat to adult corals, as long as coral reefs are able to cope with disturbances such as macroalgal proliferation. However, we anticipate severe changes under declining environmental conditions and reduced coral reef resilience.

Invertebrates are often opportunistic feeders (see Chapter 1) that due to their short life-spans and large diet range may be able to rapidly adapt to changing environmental conditions. We hypothesize that coral reefs with reduced resilience, thus comprising higher abundances of physically weakened corals, may cause *H. carunculata* to shift its generally omnivorous feeding behavior more towards scleractinian corals. This, in turn may create a negative feedback whereby the overall condition of major reef-building corals further declines and the resilience of coral reefs may be challenged. Evidence for shifting diet preferences in response to changing coral health comes from the coral-feeding wrasse *Labrichthys unilineatus* which prefers damaged to healthy coral tissue (McIlwain and Jones 1997). Similarly, several corallivorous fish species (families: Labridae, Pomacentridae, and Chaetodontidae) selectively target disease lesions and decaying tissue over adjacent healthy coral tissues (Chong-Seng et al. 2011). This behavior has the potential to increase pathogen transmission in coral reefs, and together with the putative role of *H. carunculata* as vector of pathogens, is worthy of further investigation.

Direct contact with macroalgae can lead to encroachment and mortality in scleractinian corals due to detrimental microbial and allelochemical effects (Paul et al. 2011; Rasher and Hay 2010; Smith et al. 2006); effects which can further vary in time and space (Paul and Fenical 1986). The findings in Chapter 3 confirm these observations for several common Caribbean macroalgae. Whereas *Halimeda opuntia* showed relatively moderate effects on coral mortality, *Dictyota pinnatifida* and *Cladophora* sp. caused rapid and more extensive coral tissue deterioration. Jompa and McCook (2003) found similar, species-specific variability in the degree of macroalgae-induced coral mortality during a long-term (> 1 yr) monitoring of coral-algal competition with *Porites cylindrica*. Also, Rasher and Hay (2010) confirm similar trends in that *H. opuntia* and *D. pinnatifida* exhibit very different allelochemical effects and abilities to bleach scleractinian corals. Importantly, we also found evidence for disease-like mortality patterns to occur after prolonged coral-algal interaction regardless of macroalgal species. While *H. opuntia* is known as reservoir for coral disease pathogens (Nugues et al. 2004), it seems worthwhile to investigate the microbial characteristics of those macroalgal species.

Evidence prevails that the replacement of corals by macroalgae generally followed coral mortality due to external disturbances rather than caused that mortality by competitive overgrowth (Diaz-Pulido et al. 2009; McCook et al. 2001). Clearly, macroalgal competition does evolve gradually, and the alarming findings in Chapter 3 may over-estimate natural effects, since 1) corals normally have more time to adapt to adjacent competitors, and 2) macroalgae are often exposed to strong seasonality, preventing infinite overgrowth of massive reef building corals (Ateweberhan et al. 2006; Ferrari et al. 2012; Haas et al. 2010). Coral-algal interactions should generally be addressed on a species-to-species level, since *Montastraea faveolata* has been shown to exhibit a high susceptibility to macroalgal overgrowth (Lirman 2001); also, competitive abilities of Caribbean corals against macroalgal proliferation can vary significantly among coral taxa, such as demonstrated for interaction processes with *Lobophora variegata* (Nugues and Bak 2004).

This thesis also revealed synergistic effects between two prominent stressors of coral reefs, based on indirect interaction processes among fundamentally different groups of organisms (Chapter 5). Such processes, here we identified ‘apparent competition’ (Holt 1976) and ‘habitat facilitation’ (Wootton 1994), are of utmost relevance for ecosystem functioning, since they have the potential to shape and change coral community structure (Bruno et al. 2003; Bertness and Callaway 1994; Menge 1985). The competition of macroalgae and corals for space, predation of fireworms on corals, and habitat facilitation for macroalgae and fireworms by extended space and/or shelter availability underline the complexity of community organization in marine ecosystems. Similar examples for intricate, indirect processes have been demonstrated for seaweeds, highlighting their structuring role in rocky intertidal habitats (Bertness 1999). Seaweeds can ameliorate harsh physical conditions, leading to increased species diversity underneath the algal canopy and consequent predation pressure on understory organisms such as barnacles and mussels which in return increase in abundance due to habitat modification (Menge and Sutherland 1976). Equally, the removal of seaweeds or predators from intertidal ecosystems can lead to the out-competition of other taxa by the dominant mussel *Mytilus californicus* (Paine 1977).

Interaction processes can be hidden from view, e.g. underneath algal canopies, or conversely, create clearly visible responses. Dulvy et al. (2004) illustrated such an obvious interaction cascade between overfishing, corallivory, and coral community structure. Increased overfishing (i.e. predator removal) of coral reefs along an island gradient led to the explosion of *A. planci*, which in turn caused large-scale structural changes including a 35 % decline in reef-building corals in the most intensively fished areas. Clearly, coral reef resilience is depending on a multitude of stressors: Bleaching and disease events (Ostrander et al. 2000; Sutherland et al. 2004), naturally occurring catastrophes and storms (Hughes 1994), as well as herbivore overfishing, predator removal (Jackson et al. 2001; Mumby et al. 2006) and eutrophication (Lapointe et al. 2004; Smith et al. 1981; Tomascik 1991). Identifying complex interaction patterns, whether on organism level or on

global scale between major stressors, is crucial to predict how ecosystems such as coral reefs may respond towards disturbance. Furthermore, such complex interactions highlight the importance of using multiple-stressor experimental approaches whenever possible.

Bruno et al. (2009) demonstrated that there are fewer macroalgae-dominated reefs than assumed worldwide; yet, he confirms the critical and worsening situation Caribbean reefs are facing today (Done 1992; Hughes 2004). Roff and Mumby (2012) attribute this disparity of global coral reef resilience to a variety of factors predisposing the Caribbean to its low resilience compared to Indo-Pacific reefs. Coral reefs in the Caribbean basin experience faster rates of macroalgal growth and higher rates of algal recruitment; furthermore, they inherently suffer from basin-wide iron-enrichment of algal growth from aeolian dust and are constrained by lower herbivore biomass and diversity. Littler et al. (2006) state the following: *'Due to the sensitive nature of direct/indirect and stimulating/limiting interacting factors, coral reefs are particularly vulnerable to anthropogenic reversal effects that decrease top-down controls and, concomitantly, increase bottom-up controls, dramatically altering ecosystem resiliencies.'* Roff and Mumby (2012) speculate that herbivore control may not suffice *per se* for controlling macroalgal blooms in the Caribbean. Nonetheless, the control of anthropogenically derived eutrophication, overfishing and the maintenance of herbivore biomass should be of highest concern for the management of Caribbean reefs, in order to prevent emerging or existing disturbances to proliferate.

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Erklärung

Gemäß §6 der Promotionsordnung der Universität Bremen für die mathematischen, natur- und ingenieurwissenschaftlichen Fachbereiche vom 14. März 2007 versichere ich, dass die Arbeit mit dem Titel

„The role of macroalgae and the corallivorous fireworm *Hermodice carunculata* on coral reef resilience in the Caribbean“

1. ohne unerlaubte fremde Hilfe selbständig verfasst und geschrieben wurde
2. keine anderen als die angegebenen Quellen und Hilfsmittel benutzt wurden
3. die den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht wurden
4. es sich bei den von mir abgegebenen Arbeiten um 3 identische Exemplare handelt.

Bremen, 6. September 2012

Alexander Wolf