The use of histology, molecular techniques, and *ex situ* feeding experiments to investigate the feeding behavior of the coral reef predator *Hermodice carunculata,* the bearded fireworm

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at George Mason University

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Spring Semester 2009
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DEDICATION

To the late Dr. Joan Marsden, for her inspiration as a female scientist before her time
To Dr. Suzanne Dorsey, for capturing my imagination and believing in my potential
To the late Dr. Ronald Edwards, for your ever-present patience, encouragement, and advice
To my Mom, Dad, brothers, and Michelle, for nurturing my love of marine biology and science

ACKNOWLEDGEMENTS

I would like to thank everyone who helped me ponder, plan, execute, and analyze the many research projects which have culminated in this thesis. The Dauphin Island Sea Lab, Dr. Rich Aronson, and Dr. Ryan Moody reignited my passion for field research and provided amazing opportunities to pursue and expand my love for marine ecology. My research in Barbados was made possible by the Fulbright Program of the State Department. Thank you to the people working for the program in the U.S. and for the U.S. Embassy in Barbados for your support. I would also like to thank Geoff Cook for introducing me to George Mason University. We first talked about George Mason University many years ago during a coral research cruise. Thank you Geoff for your recruiting skills, companionship, and support since my journey began at GMU. Dr. Esther Peters was an integral part in my discovery of the art of histology. Thank you for your editing skills and patience. The Bellairs Research Institute was used as base camp for Chapter 3 and other projects I conducted while living in Barbados on and off from 2001-2005. I would like to recognize the fabulous crews of M/V Fling and Spree, the fellow researchers, and the NOAA employees on the many trips to the Flower Garden Banks and the Florida Keys National Marine Sanctuaries throughout my years as a project manager and a graduate student. You all made the trips unforgettable.

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ABSTRACT

THE USE OF HISTOLOGY, MOLECULAR TECHNIQUES, AND *EX SITU* FEEDING EXPERIMENTS TO INVESTIGATE THE FEEDING BEHAVIOR OF THE CORAL REEF PREDATOR *HERMODICE CARUNCULATA,* THE BEARDED FIREWORM

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George Mason University, 2009

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Three studies on the invertebrate corallivore *Hermodice carunculata*, commonly known as the bearded fireworm, were conducted to provide baseline information on the general anatomical features, nutritional requirements, feeding behavior, and growth rates of this poorly documented reef inhabitant. Through histological techniques, the species' feeding mechanisms, digestive system, and sensory structures were studied to understand its ability to adapt to a changing reef habitat. Several previously unreported features were observed including a simple layer of cells on the outer cuticle resembling, and the size of, bacteria, a specialized tissue in the foregut, and pigment granules penetrating the ventral cuticle at the nerve cord connection. This study also documented the appearance of gut content in the digestive tract and the presence of secretory cells on the proboscis. These observations provide insight into the feeding behavior of *H. carunculata*. *Ex situ* feeding experiments were conducted in Barbados to monitor organisms' weight change in different coral reef feeding regimes as an indication of nutritional value of coral species.

During the experiments, *H. carunculata* specimens experienced a *Millepora complanata* feeding regime and a short time period in a *Montastraea annularis* feeding regime. In both experiments, the average weight change was not statistically significant, which indicates the *H. carunculata* specimens did not gain weight in the presence of *M. complanata.* These results suggest *H. carunculata* may supplement their diet with other food sources to meet nutritional requirements. Finally, during an outbreak of an unknown white syndrome on coral colonies in the Flower Garden Banks National Marine Sanctuary, field samples, including *H. carunculata* specimens associated with the coral tissue loss margins, were collected to test the use of the Polymerase Chain Reaction (PCR) and DNA fingerprinting to identify *H. carunculata* associated with tissue loss lesions. Specific *H. carunculata* primers were designed to amplify *H. carunculata* DNA in the samples. The *H. carunculata* tissue samples (positive controls) were the only samples amplified during the PCR tests. These results suggest *H. carunculata* organisms do not leave detectable amounts of DNA at foraging sites. However, future molecular tests should be conducted using other target sequences for amplification and take into considerations the sensitivity of conditions including the number of PCR cycles.

1. Introduction

Coral reefs are among the most diverse ecosystems on the planet (Huston 1985, Jackson 1991, Spalding et al. 2001, Folke et al. 2004, Wilkinson 2004, Ferner et al. 2009) and are considered the rainforests of the sea (Huston 1985, Jackson 1991, Sebens 1994, Knowlton 2001, Bellwood et al. 2004, Wilson et al. 2006, Jackson 2008, Yap 2009). Although coral reefs only occupy around 0.09% of the ocean area (Wilson et al. 2006), 25% of all animals in the ocean rely on coral reefs for survival (Spalding et al. 2001, Wilkinson 2004). These habitats are important to an enormous number of marine organisms (Sebens 1994, Chapin et al. 2000, Knowlton 2001, Pandolfi and Jackson 2006) during at least one stage of their life for shelter (Menge and Lubchenco 1981, Sano et al. 1984), food (Mumby et al. 2006, Rotjan and Lewis 2008), and breeding space (Booth and Wellington 1998, Hughes and Tanner 2000, Syms and Jones 2000, Adams and Ebersole 2002, Jones et al. 2004). The diversity of coral species offers an array of physical structures, (Ferner et al. 2009, Yap 2009) including branches (Pandolfi and Jackson 2006), plates (Huston 1985), and boulders (Edmunds and Elahi 2007), which are vital to maintain the vast community of marine biota (Crowder and Cooper 1982, Jones and Syms 1998, Syms and Jones 2000, Caley et al. 2001, Adams and Ebersole 2002, Bellwood et al. 2003, Wilson et al. 2006, Ferner et al. 2009). Live coral tissue provides a biological structure which promotes larval recruitment (Syms and Jones 2000), accretes

calcium carbonate skeleton (Hutson 1985), and provides food for corallivores (Bell and Galzin 1984).

Coral reefs are being damaged worldwide at an alarming rate (Nystrom et al. 2000, Gardner et al. 2003, Bellwood et al. 2004, Hughes et al. 2007) from a variety of threats including overfishing (Jackson et al. 2001, Pandolfi et al. 2003), storms (Hughes 1994, Aronson et al. 2004, Alvarez-Filip and Gil 2006, Cheal et al. 2008), pollution (Pastorok and Bilyard 1985, Smith et al. 2008), and elevated sea surface temperatures (Hoegh-Guldberg et al. 2007). Coral diseases can also lead to colony mortality (Bruckner and Bruckner 1997, Hughes and Tanner 2000) which may exacerbate the worldwide decline of coral reefs (Knowlton 2001, Pandolfi et al. 2003). Coral mortality has lead to unprecedented phase shifts of dominant coral species (Hughes et al. 2003, Aronson et al. 2004, Green et al. 2008), changes in reef structural features (Edmunds and Elahi 2007, Ferner et al. 2009), and increase in the number of algae-dominated reefs (Done 1992, Knowlton 1992, Knowlton 2004, Pandolfi et al. 2005). Phase shifts and mass mortality of corals can impact sessile and benthic marine organisms including the consumers of coral tissue, corallivores (Rotjan and Lewis 2008).

The impacts of coral reef decline have been studied extensively for vertebrate corallivores (Cheal et al. 2008) when compared with studies of invertebrate corallivores (Rotjan and Lewis 2005). The following chapters examined a poorly understood, direct consumer of coral tissue, the invertebrate facultative corallivore *Hermodice carunculata*, one of 160 known corallivores. *H. carunculata* is in the Class Polychaeta, Family Amphinomidae (Marsden 1962). It can be found on tropical reefs worldwide. Previous

field observations and microscopic analysis of gut content indicate that this species' diet includes coral tissue, sponges, anemones, and plant material (Marsden 1963a). Using its muscular proboscis, *H. carunculata* everts its pharynx over its prey and moves tissue into its foregut through use of its muscular pharynx (Marsden 1966). Disease events of suspect white syndrome in Flower Gardens National Marine Sanctuary (FGBNMS) and Navassa in the winters of 2004, 2005, 2006, and 2007 indicate there is a strong correlation between *H. carunculata* and suspect white syndrome (Miller and Williams 2007, Jonas personal communication). These observations suggest the *H. carunculata* populations were contributing to or the primary cause of coral tissue loss. However, limited information on this species impairs our ability to address its ability to adapt in a changing reef environment, its impact on a declining reef system, and its role in coral tissue loss and etiology of diseases including white plague (WP). *H. carunculata*'s feeding preferences, specific extraction mechanisms (i.e. the use of digestive enzymes to aid in tissue removal), and capacity to transport microbes (e.g. vector of disease) need to be well understood.

Periodic phenomena or long-term shifts in ocean conditions can affect coral reef community ecology (May 1977, Knowlton 1992, Pandolfi et al. 2003) including the abundance and biodiversity of coral species (Bythell et al. 1993, Hughes 1994, Aronson and Precht 2001). Important reef-building coral species like *Montastraea annularis* have been replaced by dominate and fast-growing species like *Porites astreoides* (Green et al. 2008). Similarly, the reef systems off of Barbados have seen a reduction in *M. annularis* and *Acropora cervicornis* (Macintyre et al. 2007). In contrast to the decrease in

scleractinian corals, the hydrocoral *Millepora complanata* remains in high densities in the shallow reefs (Lewis 2006). If the new dominant coral species are less nutritious per tissue area, this may increase the area of coral tissue loss per feeding event (Baums et al. 2003b). Also, feeding behavior of corallivores (e.g., scrapers versus grazers) may not be conducive to the newly dominant coral species morphology (Rotjan and Lewis 2008). The overall nutritional value and availability of corals as a food resource could have a detrimental effect on species (Baums et al. 2003b). If factors are not favorable for consumption of the new coral species or if coral tissue becomes unavailable (i.e., complete coral mortality), facultative corallivores may utilize alternate food resources in larger quantities, which could have compounding impacts on the reef community (Rotjan and Lewis 2008). Through histopathological methods, Chapter 2 documents the tissues, gut content, and anatomical features of eight specimens collected from the FGNMS in 2007 to develop baseline information on this species ability to detect and consume prey. The experiments in Chapter 3 examined the body weight of *H. carunculata,* in the presence of an abundant fire coral species, *M. complanata* from a system of declining scleractinian coral cover in Barbados.

Studies investigating possible causes of coral disease have had inconsistent or inconclusive findings (Ainsworth et al. 2007) including the uncertain role of corallivores in the etiology of disease (Sussman et al. 2003, Miller and Williams 2007). Effective management tools are needed to understand the correlation of microbial-induced tissue loss to corallivory (Sussman et al. 2003) and to distinguish predation scars from disease (Dalton and Godwin 2006, Miller and Williams 2007). In Chapter 4, samples of coral

tissue at the site of actively feeding organisms were tested for detectable *H. carunculata* DNA to determine the association of corallivory feeding behavior of *H. carunculata* with white syndrome tissue loss margins. Furthermore, the ability to trace *H. carunculata* DNA on predation marks could aid in the development of accurate diagnosis criteria for predation-induced versus microbial-induced tissue loss.

As coral reefs decline and distribution of coral species change, the impacts on reef inhabitants which are dependent on coral reef complexity and live coral tissue need to be understood (Sano et al. 1984, Chapin et al. 2000, Syms and Jones 2000, Booth and Beretta 2002, Samways 2005, Rotjan and Lewis 2008). Basic information is not available for some corallivores (Rotjan and Lewis 2008) including the invertebrate, facultative corallivore, *H. carunculata.* The observations in the following chapters provide fundamental information on the sensory structures, feeding behavior, and diagnosis predation patterns of *H. carunculata* which are critical in determining the impact of a deteriorating reef system on this coral reef dweller and its prey, including threatened coral species.

Literature Cited

Adams AJ, Ebersole JP (2002) Use of back-reef and lagoon habitats by coral reef fishes. Mar Ecol-Prog Ser 228:213-226

Ainsworth TD, Kramasky-Winter E, Loya Y, Hoegh-Guldberg O, Fine M (2007) Coral disease diagnostics: What's between a plague and a band? Appl Environ Microbiol 73:981-992

Alvarez-Filip L, Gil I (2006) Effects of Hurricanes Emily and Wilma on coral reefs in Cozumel, Mexico. Coral Reefs 25:583-583

Aronson RB, MacIntyre IG, Wapnick CM, O'Neill MW (2004) Phase shifts, alternative states, and the unprecedented convergence of two reef systems. Ecology 85:1876-1891

Aronson RB, Precht WF (2001) White-band disease and the changing face of Caribbean coral reefs. Hydrobiologia 460:25-38

Baums IB, Miller MW, Szmant AM (2003b) Ecology of a corallivorous gastropod, *Coralliophila abbreviata*, on two scleractinian hosts. II. Feeding, respiration and growth. Mar Biol 142:1093-1101

Bellwood DR, Hoey AS, Choat JH (2003) Limited functional redundancy in high diversity systems: resilience and ecosystem function on coral reefs. Ecol Lett 6:281-285

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

Booth DJ, Beretta GA (2002) Changes in a fish assemblage after a coral bleaching event. Mar Ecol-Prog Ser 245:205-212

Booth DJ, Wellington G (1998) Settlement preferences in coral-reef fishes: Effects on patterns of adult and juvenile distributions, individual fitness and population structure. Blackwell Science, p 274-279

Bruckner AW, Bruckner RJ (1997) Outbreak of coral disease in Puerto Rico. Coral Reefs 16:260

Bythell JC, Gladfelter EH, Bythell M (1993) Chronic and catastrophic natural mortality of 3 common Caribbean reef corals. . Coral Reefs 12:143-152

Caley MJ, Buckley KA, Jones GP (2001) Separating ecological effects of habitat fragmentation, degradation, and loss on coral commensals. Ecology 82:3435-3448

Chapin FS, Zavaleta ES, Eviner VT, Naylor RL, Vitousek PM, Reynolds HL, Hooper DU, Lavorel S, Sala OE, Hobbie SE, Mack MC, Diaz S (2000) Consequences of changing biodiversity. Nature 405:234-242

Cheal A, Wilson SK, Emslie MJ, Dolman A, Sweatman H (2008) Responses of reef fish sommunities to coral declines on the Great Barrier Reef. Mar Ecol-Prog Ser 372:211-223

Crowder LB, Cooper WE (1982) Habitat structural complexity and the interaction between bluegills and their prey Ecology 63:1802-1813

Dalton SJ, Godwin S (2006) Progressive coral tissue mortality following predation by a corallivorous nudibranch (*Phestilla* sp.). Coral Reefs 25:529-529

Done TJ (1992) Phase shifts in coral reef communities and their ecological significance Hydrobiologia 247:121-132

Edmunds PJ, Elahi R (2007) The demographics of a 15-year decline in cover of the Caribbean reef coral *Montastraea annularis*. Ecol Monogr 77:3-18

Emslie MJ, Cheal AJ, Sweatman H, Delean S (2008) Recovery from disturbance of coral and reef fish communities on the Great Barrier Reef, Australia. Mar Ecol-Prog Ser 371:177-190

Ferner MC, Smee DL, Weissburg MJ (2009) Habitat complexity alters lethal and non-lethal olfactory interactions between predators and prey. Mar Ecol-Prog Ser 374:13-22

Folke C, Carpenter S, Walker B, Scheffer M, Elmqvist T, Gunderson L, Holling CS (2004) Regime shifts, resilience, and biodiversity in ecosystem management. Annu Rev Ecol Evol Syst 35:557-581

Gardner TA, Cote IM, Gill JA, Grant A, Watkinson AR (2003) Long-term region-wide declines in Caribbean corals. Science 301:958-960

Green DH, Edmunds PJ, Carpenter RC (2008) Increasing relative abundance of *Porites astreoides* on Caribbean reefs mediated by an overall decline in coral cover. Mar Ecol-Prog Ser 359:1-10

Hoegh-Guldberg O, Mumby PJ, Hooten AJ, Steneck RS, Greenfield P, Gomez E, Harvell CD, Sale PF, Edwards AJ, Caldeira K, Knowlton N, Eakin CM, Iglesias-Prieto R,

Muthiga N, Bradbury RH, Dubi A, Hatziolos ME (2007) Coral reefs under rapid climate change and ocean acidification. Science 318:1737-1742

Hughes TP (1994) Catastrophes, phase shifts, and large scale degradation of a Caribbean coral reef. Science 265:1547-1551

Hughes TP, Baird AH, Bellwood DR, Card M, Connolly SR, Folke C, Grosberg R, Hoegh-Guldberg O, Jackson JBC, Kleypas J, Lough JM, Marshall P, Nystrom M, Palumbi SR, Pandolfi JM, Rosen B, Roughgarden J (2003) Climate change, human impacts, and the resilience of coral reefs. Science 301:929-933

Hughes TP, Rodrigues MJ, Bellwood DR, Ceccarelli D, Hoegh-Guldberg O, McCook L, Moltschaniwskyj N, Pratchett MS, Steneck RS, Willis B (2007) Phase shifts, herbivory, and the resilience of coral reefs to climate change. Curr Biol 17:360-365

Hughes TP, Tanner JE (2000) Recruitment failure, life histories, and long-term decline of Caribbean corals. Ecology 81:2250-2263

Huston MA (1985) Patterns of species diversity on coral reefs. Annu Rev Ecol Syst 16:149-177

Jackson JBC (1991) Adaptation and diversity of reef corals. Bioscience 41:475-482

Jackson JBC (2008) Ecological extinction and evolution in the brave new ocean. Proc Natl Acad Sci U S A 105:11458-11465

Jackson JBC, Kirby MX, Berger WH, Bjorndal KA, Botsford LW, Bourque BJ, Bradbury RH, Cooke R, Erlandson J, Estes JA, Hughes TP, Kidwell S, Lange CB, Lenihan HS, Pandolfi JM, Peterson CH, Steneck RS, Tegner MJ, Warner RR (2001) Historical overfishing and the recent collapse of coastal ecosystems. Science 293:629-638

Jones GP, McCormick MI, Srinivasan M, Eagle JV (2004) Coral decline threatens fish biodiversity in marine reserves. Proc Natl Acad Sci U S A 101:8251-8253

Jones GP, Syms C (1998) Disturbance, habitat structure and the ecology of fishes on coral reefs. Austral Science 23:287-297

Knowlton N (1992) Thresholds and multiple stable states in coral reef community dynamics America Zoology 32:674-682

Knowlton N (2001) The future of coral reefs. Natl Acad Sciences, p 5419-5425

Knowlton N (2004) Multiple "stable" states and the conservation of marine ecosystems. Prog Oceanogr 60:387-396

Lewis JB (2006) Biology and ecology of the hydrocoral *Millepora* on coral reefs. In: Advances in Marine Biology, Vol 50, Vol 50. Academic Press Ltd, London, p 1-55

Macintyre IG, Glynn PW, Toscano MA (2007) The demise of a major *Acropora palmata* bank-barrier reef off the southeast coast of Barbados, West Indies. Coral Reefs 26:765-773

Marsden JR (1962) A coral-eating polyachaete. Nature 193:598

Marsden JR (1963a) A Preliminary Report on Digestive Enzymes of *Hermodice carunculata*. Canadian Journal of Zoology/Revue Canadien de Zoologie 41:159-164

Marsden JR (1966) The coelomocytes of *Hermodice carunculata* (Polychaeta: Amphimomidae) in relation to digestion and excretion. Canadian Journal of Zoology/Revue Canadien de Zoologie 44:377-389

May RM (1977) Thresholds and multiple stable states in coral reef community dynamics. Nature 269:471-477

Menge BA, Lubchenco J (1981) Community organization in temperate and tropical rocky intertidal habitats - prey refuges in relation to consumer pressure gradients Ecol Monogr 51:429-450

Miller MW, Williams DE (2007) Coral disease outbreak at Navassa, a remote Caribbean island. Coral Reefs 26:97-101

Mumby PJ, Dahlgren CP, Harborne AR, Kappel CV, Micheli F, Brumbaugh DR, Holmes KE, Mendes JM, Broad K, Sanchirico JN, Buch K, Box S, Stoffle RW, Gill AB (2006) Fishing, trophic cascades, and the process of grazing on coral reefs. Science 311:98-101

Nystrom M, Folke C, Moberg F (2000) Coral reef disturbance and resilience in a human-dominated environment. Trends Ecol Evol 15:413-417

Pandolfi JM, Bradbury RH, Sala E, Hughes TP, Bjorndal KA, Cooke RG, McArdle D, McClenachan L, Newman MJH, Paredes G, Warner RR, Jackson JBC (2003) Global trajectories of the long-term decline of coral reef ecosystems. Science 301:955-958

Pandolfi JM, Jackson JBC (2006) Ecological persistence interrupted in Caribbean coral reefs. Ecol Lett 9:818-826

Pandolfi JM, Jackson JBC, Baron N, Bradbury RH, Guzman HM, Hughes TP, Kappel CV, Micheli F, Ogden JC, Possingham HP, Sala E (2005) Ecology - Are US coral reefs on the slippery slope to slime? Science 307:1725-1726

Pastorok RA, Bilyard GR (1985) Effects of sewage pollution on coral reef communities Mar Ecol-Prog Ser 21:175-189

Rotjan RD, Lewis SM (2008) Impact of coral predators on tropical reefs. Mar Ecol-Prog Ser 367:73-91

Samways MJ (2005) Breakdown of butterflyfish (Chaetodontidae) territories associated with the onset of a mass coral bleaching event. Aquatic Conservation: Marine and Freshwater Ecosystems 15:S101-S107

Sano M, Shimizu M, Nose Y (1984) Changes in structure of coral-reef fish communities by destruction of hermatypic corals - observational and experimental views. Pacific Science 38:51-79

Sebens KP (1994) Biodiversity of coral reefs - what are we losing and why. Amer Soc Zoologists, p 115-133

Smith TB, Nemeth RS, Blondeau J, Calnan JM, Kadison E, Herzlieb S (2008) Assessing coral reef health across onshore to offshore stress gradients in the US Virgin Islands. Mar Pollut Bull 56:1983-1991

Spalding M, Ravilious C, Green E (2001) World Atlas of Coral Reefs, Vol. University of California Press, Berkeley

Sussman M, Loya Y, Fine M, Rosenberg E (2003) The marine fireworm *Hermodice carunculata* is a winter reservoir and spring-summer vector for the coral-bleaching pathogen *Vibrio shiloi*. Environmental Microbiology, 2003 Apr, 5(4):250-5

Syms C, Jones GP (2000) Disturbance, habitat structure, and the dynamics of a coral-reef fish community. Ecology 81:2714-2729

Wilkinson C (ed) (2004) Status of coral reefs of the world, Vol 1. Global Coral Reef Monitoring Network and Australian Institute of Marine Science, Townesville, Australia

Wilson SK, Graham NAJ, Pratchett MS, Jones GP, Polunin NVC (2006) Multiple disturbances and the global degradation of coral reefs: are reef fishes at risk or resilient? Glob Change Biol 12:2220-2234

Yap HT (2009) Local changes in community diversity after coral transplantation. Mar Ecol-Prog Ser 374:33-41

2. A histological analysis of the invertebrate corallivore *Hermodice carunculata*

Abstract

The diversity of coral species provides habitat niches through their physical structure and live tissue which are essential for many marine organisms. Given the alarming rate of worldwide coral reef decline, many studies have questioned the consequences for marine species which rely on the physical and biological structure of coral reef diversity for shelter and food. The association of the reef community with coral structure is significant, but may be different for dwellers like herbivores and omnivores versus corallivores and coral-nesting fishes. The functional group corallivores, specifically the invertebrate *Hermodice carunculata*, is of particular interest in this study. Of the known 160 corallivores, *H. carcunculata* is one of the 5 corallivorous invertebrate phyla. *H. carunculata* is poorly understood despite its documented vector capabilities and its association with coral tissue loss margins. Through histopathological techniques, this study provides an overview of *H. carunculata*'s key anatomical features including the sensory structures, digestive system, and feeding apparatus. Previously unreported structures were also observed including a thin layer of coccoid cells on the outside of its cuticle which may have ecological implications.

Introduction

Coral reef species contribute to the primary physical structure of reef communities (Newman et al. 2006, Pandolfi and Jackson 2006). Coral species diversity results in habitat complexity which influences the abundance, distribution, and survivorship of many marine organisms (Adams and Ebersole 2002). Various coral reef dwellers use the 3-dimensional relief provided by coral species for physical protection and breeding space (Connell and Kingsford 1998, Lindahl et al. 2001, Cabaitan et al. 2008, Yap 2009). The density of branch-dwelling fishes has been correlated with the abundance of coral branches (Sano et al. 1984, Syms and Jones 2000, Yap 2009). The 3-dimensional relief of coral species has also been linked to roving herbivores (Adams and Ebersole 2002, Mumby et al. 2006). Reef substrates covered with live coral tissue have more diverse and abundant marine communities than in areas with bare or dead substrate (Bell and Galzin 1984, Yap 2009).

The living component of coral, the animal tissue with symbiotic algae, is important for reef dwellers (Bell and Galzin 1984, Carr and Hixon 1997, Hughes and Tanner 2000). Live coral tissue is a more suitable substrate to settle on for many marine organisms (Carr and Hixon 1997, Yap 2009). Without the live tissue, the coral substrate issusceptible to algal overgrowth (Yap 2004, Pandolfi et al. 2005, Smith et al. 2006), erosion (Lindahl et al. 2001, Wilson et al. 2006), and wave energy (Aronson et al. 2004, Alvarez-Filip and Gil 2006, Smith et al. 2008). Furthermore, the calcium carbonate skeleton accreted by live coral colonies increases the physical structures of the reef, enhancing habitat complexity (Hoegh-Guldberg et al. 2007, Yap 2009). While coral

species diversity is maintained by occasional disturbances (Wilson et al. 2006), the increased intensity and frequency of local and global disturbances have caused abnormal loss of live coral tissue (Wilkinson 2004, Pratchett et al. 2006) and degradation of reef structures (Alvarez-Filip and Gil 2006) leading to decreased habitat complexity (Done 1992, Hughes et al. 2007) and increased threats to reef inhabitants (Menge and Lubchenco 1981, Newman et al. 2006).

Specific responses of reef inhabitants to coral reef disturbances are dependent on their reliance on reefs for food and shelter (Booth and Wellington 1998, Berumen et al. 2005, Cabaitan et al. 2008). Decline in coral tissue abundance has been shown to most severely affect obligate corallivorous fishes (Rotjan and Lewis 2005, Wilson et al. 2006), resulting in a shift to communities dominated by omnivores and facultative corallivores (Sano et al. 1984, Wilson et al. 2006, Pratchett et al. 2008). Also, reef dwelling fishes associated with specific coral species are consistently negatively affected after habitat disturbances (Cheal et al. 2008). The negative effects of long-term coral decline on reef dwellers, specifically reef fishes, can include a reduction in energy reserves (Pratchett et al. 2004), growth rates (Kokita and Nakazono 2001), and recruitment (Jones et al. 2004); all of which can influence future adult populations (Jones et al. 2004, Wilson et al. 2006). An unpublished study by this author revealed a reduction in average body length in populations of the corallivorous invertebrate *H. carunculata* on Caribbean reefs off Barbados. Marsden (personal communication) found the average body length of this species on Barbados reefs to be 8 cm in 1965. In 2002, the average body length of the same population was 4.5 cm (Lewis, unpublished data). Between 1965 and 2002,

Barbados reefs experienced mass mortality and reduced cover of live coral tissue (Pandolfi and Jackson 2006). However, the correlation between live coral cover and body length of this corallivore species has not been established. Therefore, to understand the relationship between coral reef decline and the diet and habitat requirements of functional groups, like corallivores (Rotjan and Lewis 2005), it is imperative to determine the corallivorous species' susceptibility to physical and biological changes in coral reef ecosystems (Bell and Galzin 1984, Jones et al. 2004, Berumen et al. 2005, Bellwood et al. 2006, Newman et al. 2006, Hughes et al. 2007).

Vertebrates and invertebrates are among the 116 known corallivores (Rotjan and Lewis 2008). The dietary habits and habitat diversification of the 40 species of corallivorous fishes have been established through various studies (Bell and Galzin 1984, Motta 1988, Tricas 1989, Kokita and Nakazono 2001, Pratchett et al. 2004, Berumen et al. 2005, Samways 2005, Wilson et al. 2006). However, research on invertebrate corallivores, like *H. carunculata* and *Coralliophila abbreviata*, has been very limited (Baums et al. 2003a, Bau ms et al. 2003b, Miller and Williams 2007) and the consequences of declining coral tissue cover on these coral predators and their alternative prey is relatively unknown (Lizama and Blanquet 1975, Hayes 1990, Lewis and Crooks 1996, Baums et al. 2003b). The impact of corallivores on a declining coral reef system is also not well studied (Ott and Lewis 1972, Witman 1988, Martin and Losada 1991, Sussman et al. 2003). The dramatic rate of coral reef decline and structural changes of coral reef communities contributes to a sense of urgency to establish baseline information

on coral tissue consumers (Sussman et al. 2003, Miller and Williams 2007, Rotjan and Lewis 2008).

H. carunculata is associated with shallow tropical reefs (Witman 1988, Lewis and Crooks 1996) and is known to consume tissue from a variety of coral species as well as other marine organisms such as anemones and sponges (Marsden 1963, 1963a, 1963b). However, information on the species' dietary habits including the percentage make-up of coral tissue in the overall diet in minimual (Miller and Williams 2007). Marsden (1962) provided preliminary observations on the gut contents of this organism. Though she did not observe active feeding, she found a variety of materials in the gut including plant particles, sand, nematocysts, and masses of cells resembling zooxanthellae from coral tissue (Marsden 1962). Previous studies on this organism focused on foraging habits of *H. carunculata* as it relates to the hydrocoral *Millepora complanata* (Ott and Lewis 1972, Witman 1988, Lewis and Crooks 1996), feeding habits on one type of gorgonian through *ex situ* experiments (Sussman et al. 2003), and observations of irregular feeding on tissue loss margins of *Montastraea annularis* colonies (Miller and Williams 2007). Observations on comprehensive diet composition, feeding mechanisms, and general anatomical features of *H. carunculata* are lacking in the published literature.

In the present study, *H. carunculata* specimens were examined using histological techniques and light microscopy to document baseline information on *H. carunculata* needed to understand this species' adaptability in shifting reef ecosystems. Knowledge of *H. carunculata* dietary habits and prey extraction techniques is essential to determine the consequences for (1) alternative prey as an effect of global decline of available coral

tissue and (2) the condition of the fireworm when consuming more abundant but perhaps not as nutritious food, and (3) its feeding mechanism adaptability to successfully consume other more abundant food types. Also, information on defense mechanisms and other anatomical features and coloration are important to understand this species' ability to evade predators and adjust in reef systems with degrading protective structural features.

Methods

Collection and Fixation

The specimens for this study were collected in March 2007 from the Flower Gardens Bank National Marine Sanctuary (FGBNMS). During a series of SCUBA dives on the West Bank (27°52′30.6″ latitiude and 93°48′54.1″ longitude) and the East Bank (27°54′33.0″ latitude and 93°35′59.7″ longitude), eight random specimens, not actively feeding on coral tissue, were collected and placed in separate 50 ml, sterile, polypropylene, screwcapped tubes (Falcon™ tubes – BD Biosciences) filled with sterilized seawater from the collection site. Once on the surface, all worms were removed from the Falcon™ tubes and immediately placed in a fixative solution (1 part Z-Fix concentrate [Anatech Ltd.] mixed with 4 parts 0.2 µm porosity filtered ambient seawater). They were transported to the Histology Laboratory at George Mason University. A total of ninety-two slides prepared from the eight specimens were examined.

Trimming Tissue

Each fireworm was removed from the fixative and placed on a dissecting board. All specimens were trimmed with a scalpel in sections according to Figure 2.1. Section I was the head cut, two segments past the hard section. It was cut along the midsagittal plane into a left lateral and a right lateral section. Section II had two sections IIA (anterior cross section) and IIP (posterior cross section). These sections were cut perpendicular to the axis into two 3-mm thick pieces. Section III was a longitudinal cut, 1 cm in length, along the midsagittal plane. Section IV was similar to section II and had two sections, IVA (anterior cross section) and IVP (posterior cross section). These sections were cut along the axial plane into two 3 mm pieces. Section V was a longitudinal cut of the final posterior section. This section of tissue was cut into left lateral and right lateral sections along the midsagittal plane. Due to small body lengths, three specimens had sections I-III and did not have sections IV-V. Table 2.1 shows the number of slides per section.

Trimmed tissue sections were individually wrapped in lens paper, placed in a labeled round cassette (side of tissue for microscopic viewing face down), and stored in a plastic tub filled with fixative (1 part Z-Fix concentrate [Anatech Ltd.] mixed with 4 parts 0.2 μm porosity filtered ambient seawater water).

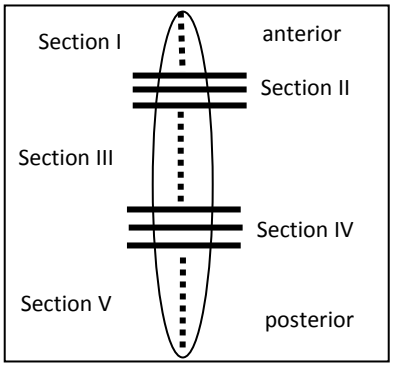


Figure 2.1 A pictorial representation of the trimmed tissue sections. The dotted lines indicate the parasaggital sections.

Table 2.1 Total number of slides analyzed for each section. Sections IVA-V were prepared for longer worms only.

Section	**Total # of Slides (HandE and Cason's)**	**Samples**
I	**14**	**All except 07-053**
IIA	**16**	**All**
IIP	**16**	**All**
III	**16**	**All**
IVA	**10**	**07-056, 07-057, 07-058, 07-059, 07-060**
IVP	**10**	**07-056, 07-057, 07-058, 07-059, 07-060**
V	**10**	**07-056, 07-057, 07-058, 07-059, 07-060**

Processing and Staining Sections

The cassettes were removed from the fixative and washed in running tap water for 15 min and then placed in a tissue processor with fresh reagents according to Peters et al. (2005). Each processor treatment step was set for 45 min.

After the completion of processing, the basket of cassettes was placed in a beaker of molten Paraplast Extra at 56 °C and transferred into a vacuum oven kept at 60 °C. Vacuum infiltration was performed manually by pumping out the air from the oven until it reached 21-22 inches (in) Hg, and vacuum was held for 1 m. Air was returned to the chamber slowly, then the vacuum pumping started again. Air pressure in the oven remained at 21-22 in Hg for 10-min before venting and removing the beaker and transferring specimens to an embedding center. The specimens were then embedded in Paraplast Xtra and sectioned at 4-µm thickness using disposable microtome blades. One section from the ribbon was placed on a labeled slide, after smoothing it on the surface of a water bath (45 °C). The slide was placed vertically for several minutes to drain water away from the wax and then placed horizontally on a slide warmer set at 45 °C for drying. This procedure was repeated for each block to obtain two slides per block.

For this study, two staining procedures were used: Mayer's hematoxylin and eosin (H&E) and Cason's aniline blue. Both used the same methodology for removing the paraffin and rehydrating the tissue. The paraffin was removed and the sections were rehydrated and dehydrated according to standard protocols (Peters et al. 2005).

After rehydration, the slides were moved to a dish containing Mayer's hematoxylin solution for 15 min followed by a water rinse for 2 min in deionized water.

For Cason's procedure, the slides were placed in Bouin's fixative as a mordant for the aniline blue dye to bind to collagen for 1 h followed by Cason's stain for 5 min and a water rinse for 3-5 s. The post-staining dehydration steps were the same for both staining procedures. The stained slides were coverslipped with Permount.

Results

Integumentary System

Epidermis and Cuticle

H. carunculata has an epidermis composed of pseudostratified columnar cells overlaid by a cuticle (Figure 2.2). The base of the epidermis rests on collagenous connective tissue (basement membrane). Melanocytes are found throughout the epidermis, varying by individual fireworm in color and range of colors (Figure 2.3). The pigment granules in Cason's-stained sections range from green, orange, red, purple, and pink. Interestingly, there are a few specimens where clusters of green granules are found in the same orientation in each segment throughout the worm (Figure 2.4). There is a uniform film lining the external surface of the collagenous cuticle (Figure 2.5). This film is a simple layer of cells resembling, and of the size of, bacteria. Circular and longitudinal muscles are found at the base of both the ventral and dorsal epidermis (Figure 2.3 and 2.4). The dorsal epidermis has a rigid appearance with frequent and small grooves while the ventral epidermis is smoother with longer grooves (compare dorsal epidermis in Figure 2.4 to ventral in Figure 2.3).

Parapodia

This species has two sets of parapodia: each consists of a notopodium (dorsal) and a neuropodium (ventral) (Figure 2.6). Both sets encase varying numbers of toxic setae. The setae are produced in epidermal follicles. One follicle forms a single seta which is lined with a cuticle and filled with pigmented granules (Figure 2.7). A chaetoblast cell forms the base of the follicle.

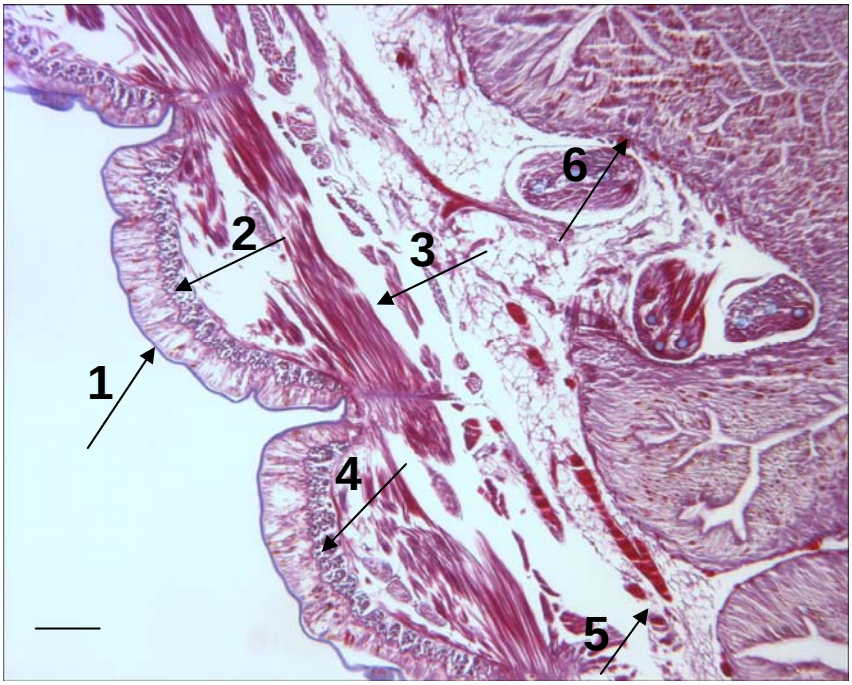


Figure 2.2 Parasaggital section, dorsal view, of the epidermis from fireworm sample 07-53 III. The epidermis rests on a basement membrane (2) and is lined with a collagenous cuticle (1). Various pigments are found within the epidermis. Longitudinal muscles (3) and circular muscles (4) run along the base of the epidermis. Adipose tissue is found throughout the coelomic cavity and is associated with blood vessels (5) and the digestive tract (6). Scale bar = 100 µm

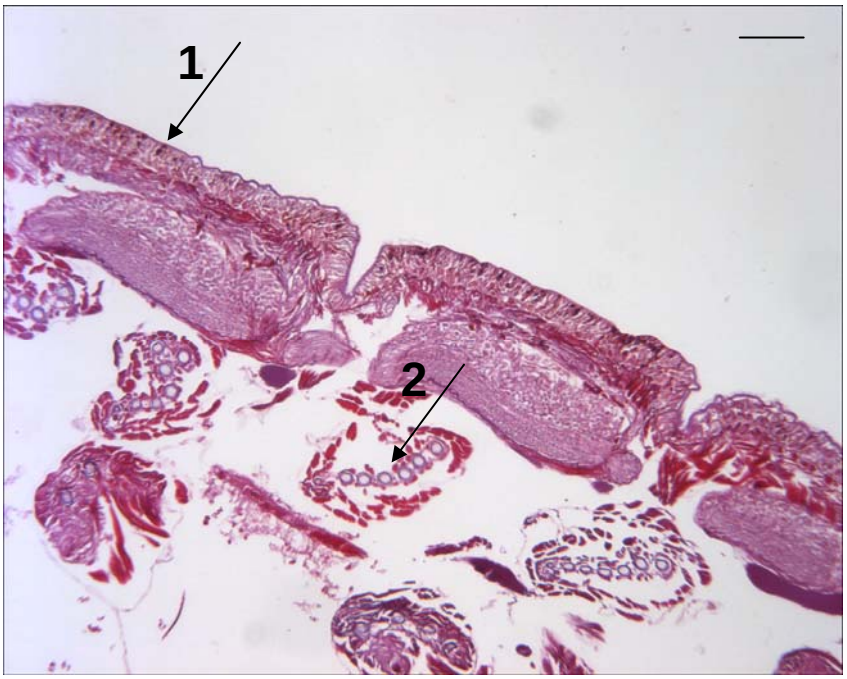


Figure 2.3 Parasaggital section, ventral view, of the fireworm sample 07-60 V. Brown granular pigments (1) are found in the dorsal epidermis. The clusters of rings are transverse sections through the bases of the hollow setae (2). Scale bar = 100 µm

Circulatory system

There are several blood vessels found in this species. The main vessel is the longitudinal dorsal blood vessel (Figure 2.11). There are also ventral vessels that surround the nerve cord. Some dorsal cross-sectional blood vessels have been observed in each segment. Blood vessels can also be found surrounding the gut cavity (Figure 2.2). Adipose tissue encompasses blood vessels (Figure 2.9).

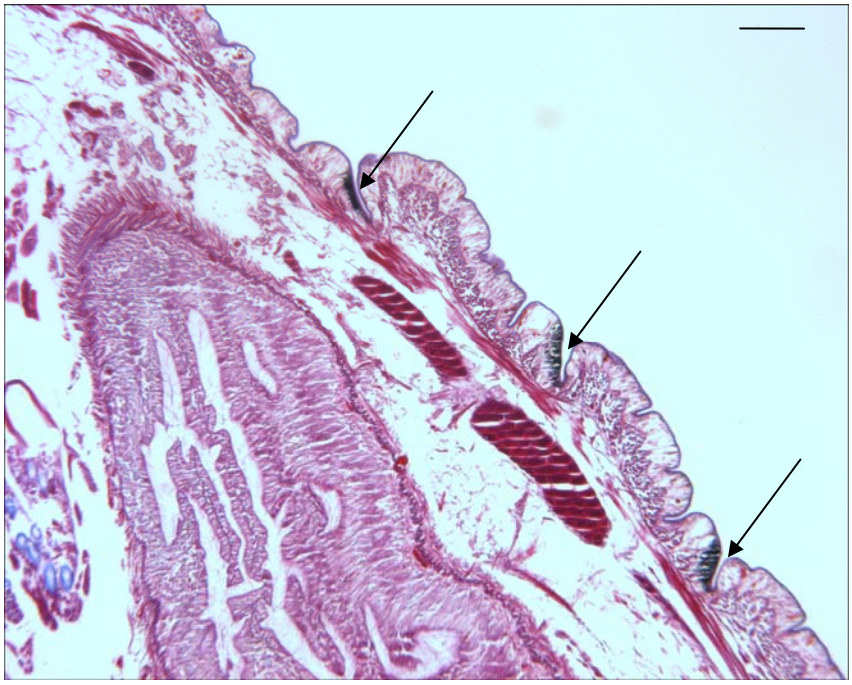


Figure 2.4 Parasaggital section, dorsal view, of the fireworm sample 07-53 III. The epidermis in this specimen has green granular pigments (black arrows) at the posterior end of each segment throughout the body. Scale bar = 100 µm

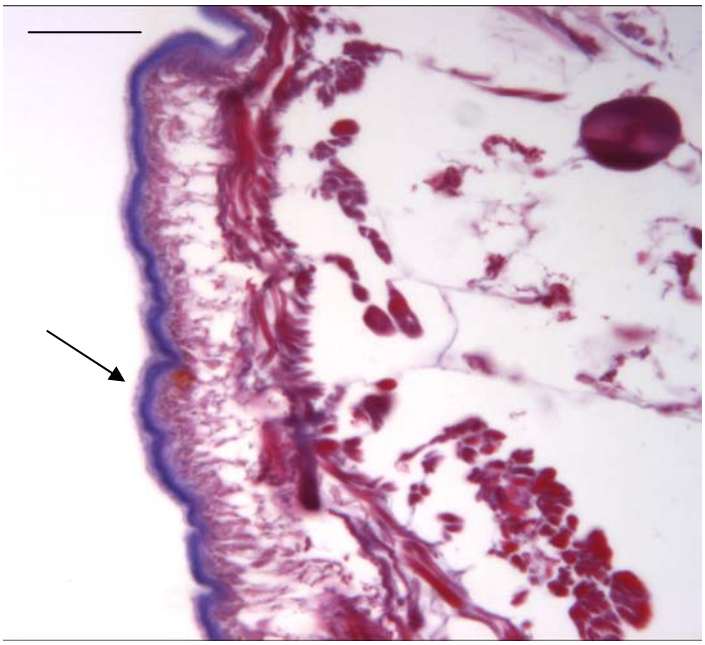


Figure 2.5 Cross-section, ventral view, of fireworm sample 07-56 IVA. Layer of cells (black arrow) are uniformly distributed on the surface of the cuticle. Scale bar = 50 µm

Figure 2.6 Side view of *Hermodice carunculata.* The two red arrows indicate the parapodia. The notopodium (1) is dorsal and the neuropodium (2) is located ventrally.

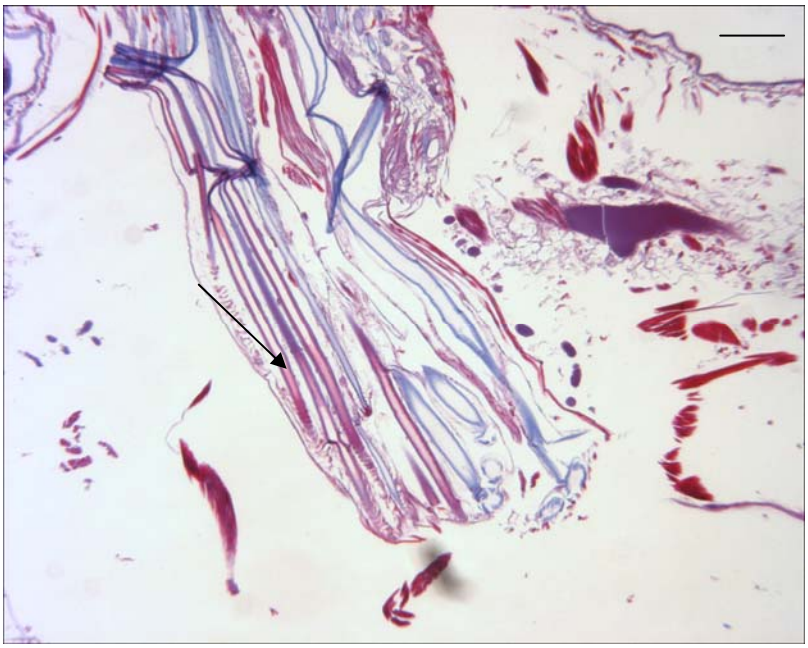


Figure 2.7 Cross-section of fireworm sample 07-58 IVP. The toxic setae have cuticle coverings and are filled with red cells (black arrow). Scale bar = 100 µm

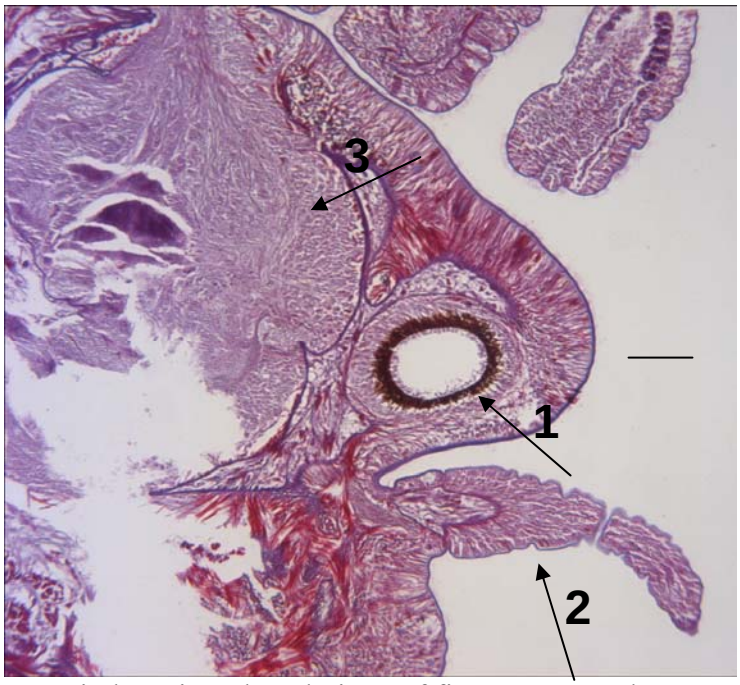


Figure 2.8 Parasaggital section, dorsal view, of fireworm sample 07-59 I. The eye (1) is a simple photoreceptor which is surrounded by brown-pigmented cells. The dorsal cirri (2) is another sensory receptor. The photoreceptor (1) and the nuchal organ are connected to the cerebral ganglion (3). Scale bar = 100 μm

Nervous System

This species has a two-lobed ventral nerve cord that runs anterior to posterior (Figure 2.12). The cord is connected to the epidermis and the pigmented granules at this connection are at a higher density than anywhere else in the body (Figure 2.12). Also, granules at this connection penetrated the cuticle (Figure 2.13). Blood vessels are found on both sides of the cord (Figure 2.11).

A cerebral ganglion is noticeable ventral and posterior to the eye (Figure 2.8). The ganglion is connected to the dorsal nuchal organ (Gardiner 1992), also known as the caruncle (Fauchald 1977) through nerve fibers (Figures 2.8 and 2.9). The cerebral

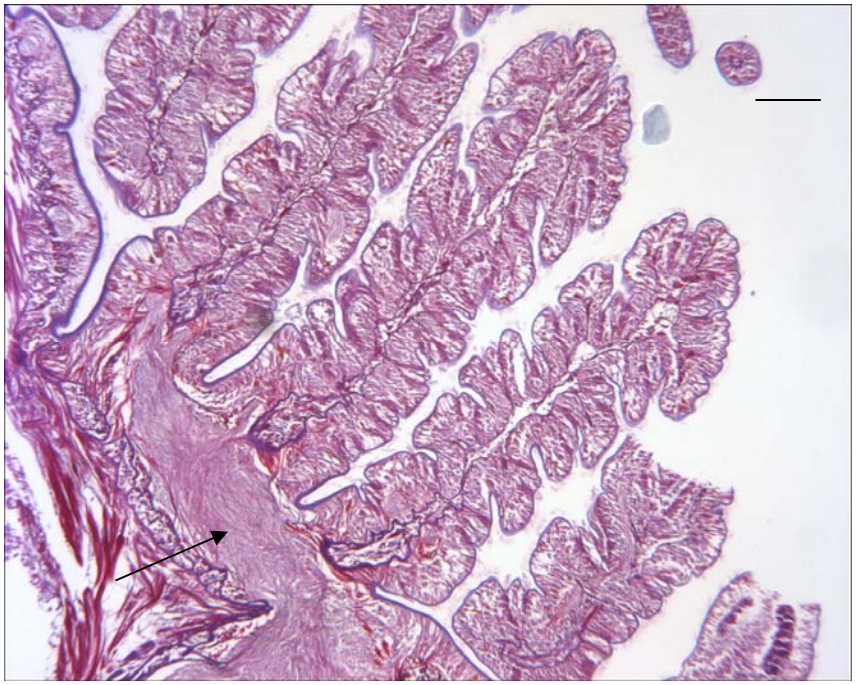


Figure 2.9 Parasaggital section, dorsal view, from fireworm sample 07-59 I. Sensory nerve fibers (black arrow) connect the nuchal organ to the cerebral ganglion. Scale bar = 100 µm

Figure 2.10 Dorsal view of *Hermodice carunculata.* The red arrow indicates the location of the buccal region, located at the most anterior part of the body.

Figure 2.11 Cross-section view of fireworm sample 07-58 IVP. The main blood vessel (2) is dorsal. Several blood vessels (4) are located around the ventral nerve cord (5). Notice the notopodium (1) and the neuropodium (3). Scale bar = 40 mm

ganglion consists of neurons and a neuropil of nueraxons and dendrites. The connection from the cerebral region and the ventral nerve cord was not observed.

Sensory Structures

H. carunculata has two eyes and each has photoreceptor cells surrounded by brown- pigmented (Cason's and H&E stains) granules (Figure 2.8). Nerve fibers are found around the pigmented granules and neurons are attached to the pigmented areas. This area may reflect light to increase photosensitivity. A second feature of this species' sensory system is the nuchal organ which is located dorsally at the anterior end of the body (Figure 2.9 and 2.10). It is a complex folded structure with the typical

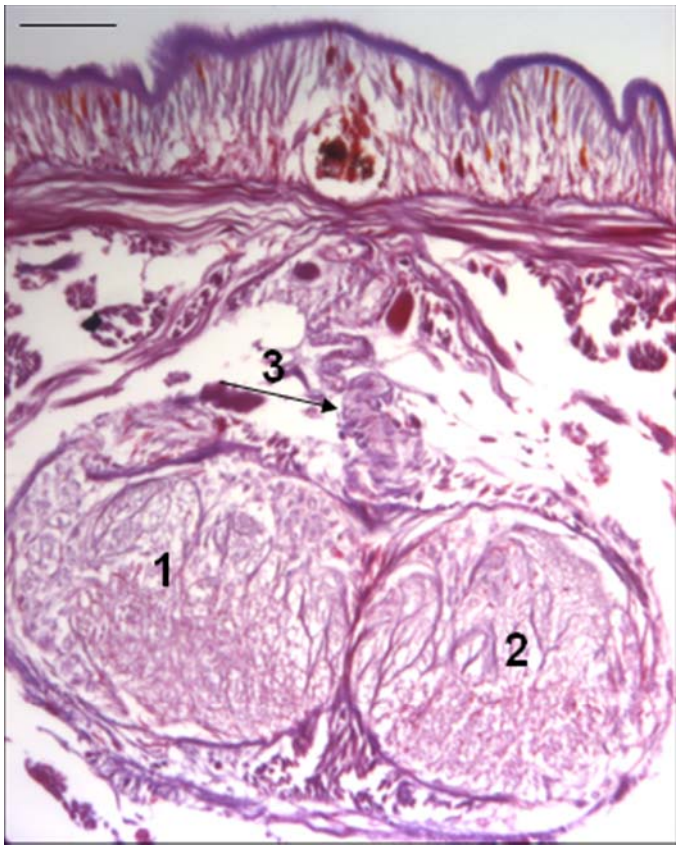


Figure 2.12 Cross-section, ventral view, from fireworm sample 07-59 IIA. The nerve cord is two lobed (1 and 2) and is connected to the epidermis (3). Scale bar = 40 µm

epidermis and covering cuticle layer. It is immediately posterior to the photoreceptor. The sensory cells connect the photoreceptor and the nuchal organ to the nerve cells (Figure 2.8) posterior to the eye.

Excretory System

A well defined excretory system was not observed in these specimens. However, literature sources (Fauchald 1977, Gardiner 1992) state that the metanephridium (which

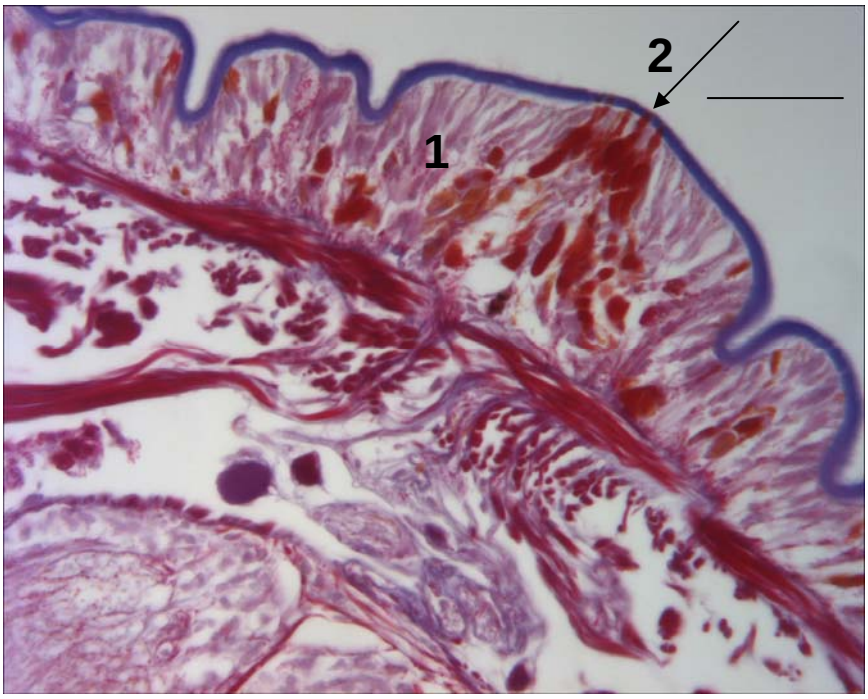


Figure 2.13 Cross-section of fireworm sample 07-59 IVP. The pigmented cells in the epidermis are at highest density where the nerve cord connects to the epidermis (1). Some of the granules from the pigmented cells penetrate the cuticle (2). Scale bar = 50 µm

includes the nephrostome, nephridial tubule, and the nephridiopore) in polychaetes may be associated with the nerve cord. Therefore, further analysis will be needed to better understand the function and association of the nerve cord with the excretory system of *H. carunculata.*

Digestive System

The digestive tract runs longitudinally from anterior to posterior and is a composed of pseudostratified columnar ciliated epithelial cells on a basement membrane (Figures 2.18-2.20).

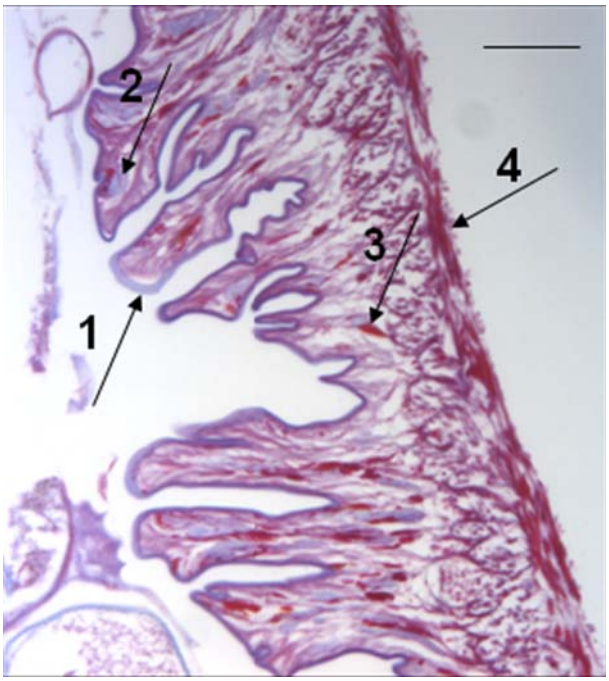


Figure 2.14 Parasaggital section of the foregut from fireworm sample 07-56 I. The foregut is lined with a thick collagenous cuticle (1), which stains blue with aniline blue. Circular muscles line the foregut (4). Granular secretory cells and mucus-secreting cells (mucocytes) are part of the pseudostratified epithelium (2 and 3). Scale bar = 50 µm

Foregut

Like many polychaetes, *H. carunculata* has an eversible proboscis. Numerous layers of longitudinal and circular muscles (varying in thickness) are observed in this anterior region which supports its ability to extract food in the absence of jaws. Also, the pseudostratified columnar epithelium in this region is lined with a thick cuticle which may function to protect this region during eversion of the pharynx when feeding (Figure 2.14). The proboscis has large, dense pockets of granular secretory cells ranging in colors from red to orange, purple, and yellow (Cason's stain; purple and red in H&E stain)

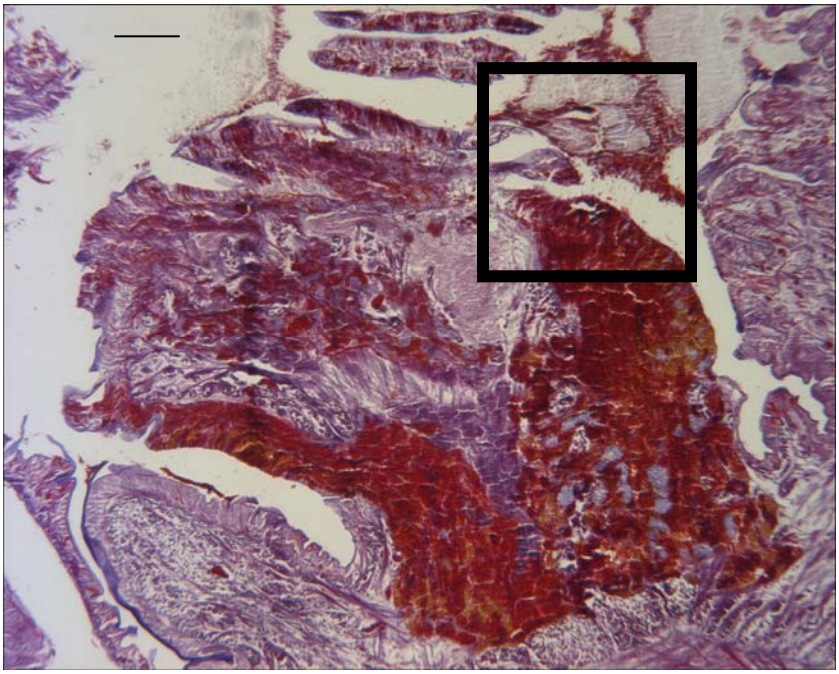


Figure 2.15 Parasaggital image of the foregut from fireworm sample 07-057 I. The proboscis contains thick clustering of red, pink, orange, and purple-staining (with Cason's procedure) granular secretory cells, possibly used during feeding. The cuticle is very thin in the sections. The black box indicates the section captured at higher magnification in Figure 2.16. Scale bar = 100 μm

(Figure 2.15 and 2.16). These cells are not found anywhere else in the body. These secretory cells may take part in the feeding mechanism to extract food.

The foregut has a cuticle lining the epithelium (Figure 2.14). The cuticle thins and disappears posterior in sections IIA and IIP. Secretory cells are present; however, there appears to be no interaction between food particles and the epithelium in this region. Given its location in the most anterior portion of the gut, this cuticle-lined epithelium is probably the pharynx. A cuticle-lined epithelium in this section of the gut will function as protection during feeding when this region is exposed and potentially vulnerable to foreign objects.

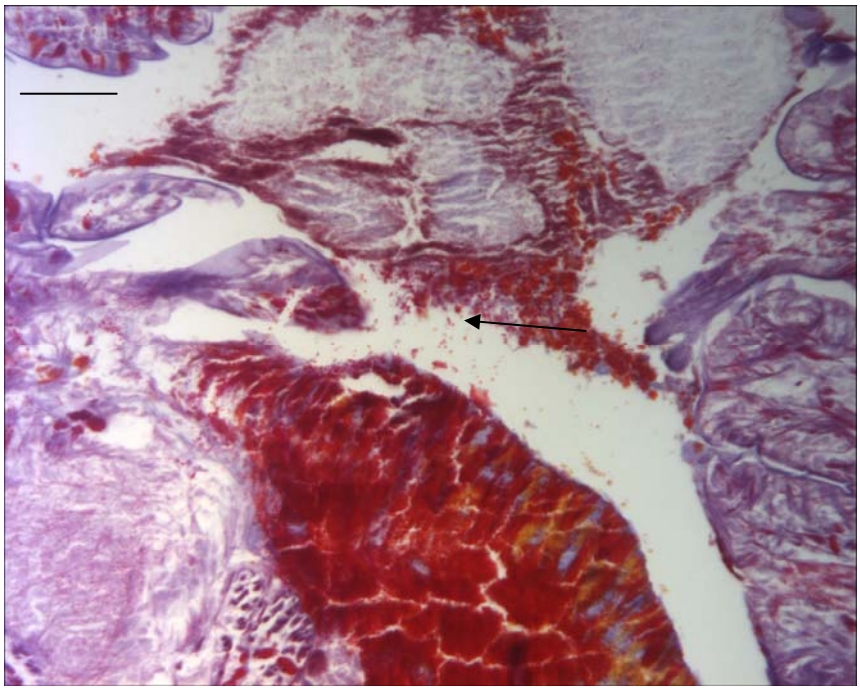


Figure 2.16 Close-up image of Figure 1.15. The black arrow indicates the release of the secretory granules into the gut cavity. These granules may function to break down food during feeding and after ingestion. Scale bar = 50 µm

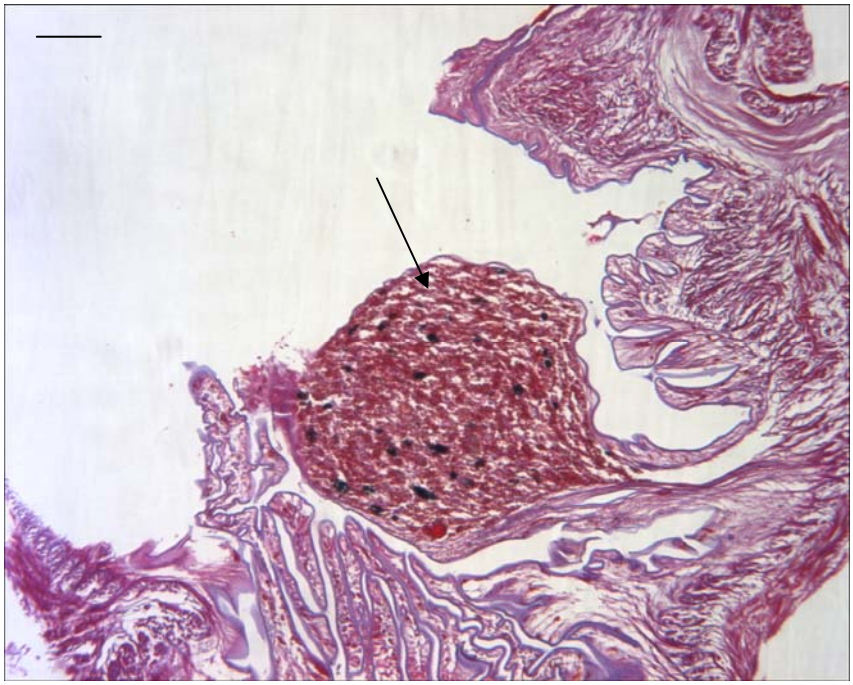


Figure 2.17 Parasaggital section from fireworm sample 07-56 I. The black arrow indicates a specialized tissue. It is surrounded by a cuticle and has blood vessels and pigmented cells (Cason's stain shown). Its function is unclear. Scale bar = 100 µm

Interestingly, there is a specialized tissue in the anterior section of the epithelium (Figure 2.17). The structure is lined with a cuticle and comes out into the gut cavity from the epithelium on a stalk. It is located within the foregut near the proboscis and is filled with brown and red cells (Cason's stain). In the H&E stained sections, the cells are purple and pink. Blood vessels are present and epithelium cells are absent. Its function is unknown.

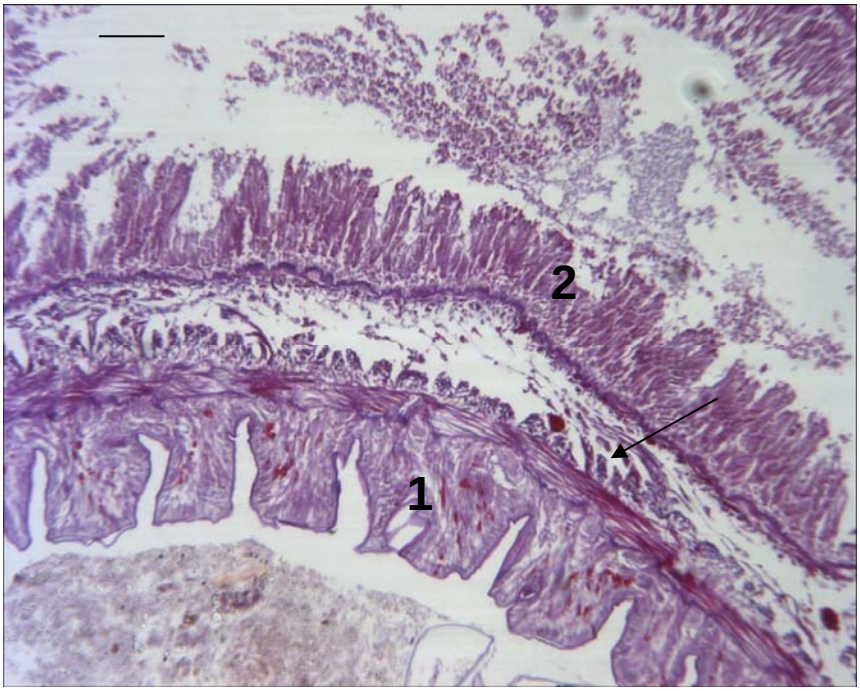


Figure 2.18 Cross-section of the foregut (1) and more posterior digestive tract (2) from fireworm sample 07-59IIA. The foregut is lined with a thick blue cuticle and the epithelium contains red granular secretory cells and mucocytes. The digestive tract epithelium (2) is not lined with a cuticle and there is an absence of cilia and the large secretory cells found in the foregut and proboscis. The black arrows indicate a longitudinal muscle. Scale bar = 100 µm

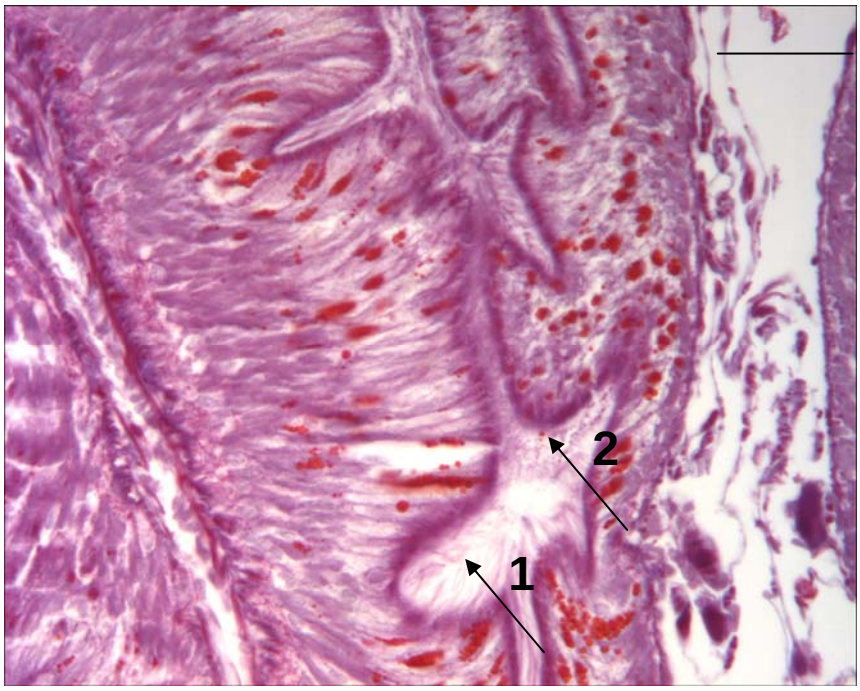
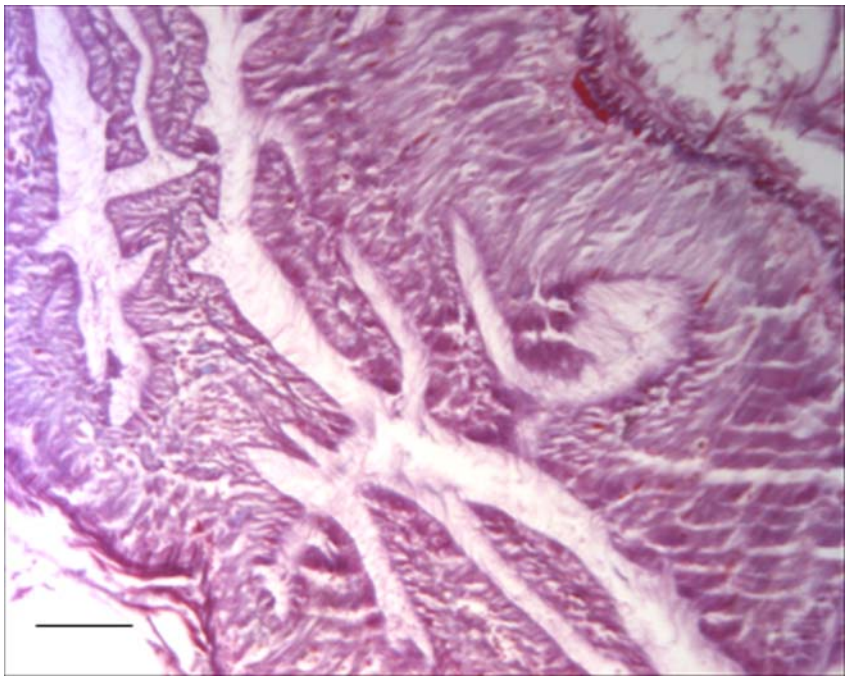


Figure 2.19 Parasaggital section of the digestive tract posterior from fireworm sample 07-055 III. Cilia are present on the apical surfaces of these columnar cells (1). The secretory cells are present in the epithelium and granules are released into the gut cavity as indicated by the black arrow (2). Scale bar = 50 μm

Midgut

The midgut is anterior to the foregut and contains the intestine which has a microvillous appearance. The intestine epithelium contains different cells, including gland cells that appear to produce zymogen granules, which are discharged into the lumen (Figures 2.16 and 2.19). Within the epithelium, mucocytes are found in sections III and IV. Both circular and longitudinal muscle layers encompass the wall.

Cells are ciliated with varying degrees throughout the gut. The longer, more pronounced cilia are found in the posterior epithelium, which also lacks granular secretory cells (Figure 2.20). Shorter cilia are found in the anterior portion of the gut, where there is also



F**igure 2.20** Parasaggital section of the most posterior gut cavity from fireworm sample 07-053 III. The epithelium is unlined and the length and density of cilia is higher in this region of the digestive tract than in other regions. Also, few secretory cells are present. Scale bar = 50µm

a higher density of granular secretory cells (Figure 2.19). Furthermore, higher densities of granular secretory cells are found within the gut cavity in the anterior section of the gut (sections I-II) (Figure 2.16). The disparity between anterior and posterior epithelium in relation to cilia and suspected zymogens may be an indication that the main site of digestion is the anterior intestine. The long cilia in the posterior epithelium function to transport the remaining materials to the rectum.

The feeding behavior for these specimens was not observed at the time of collection. Also, these specimens were collected at different sites on different days.

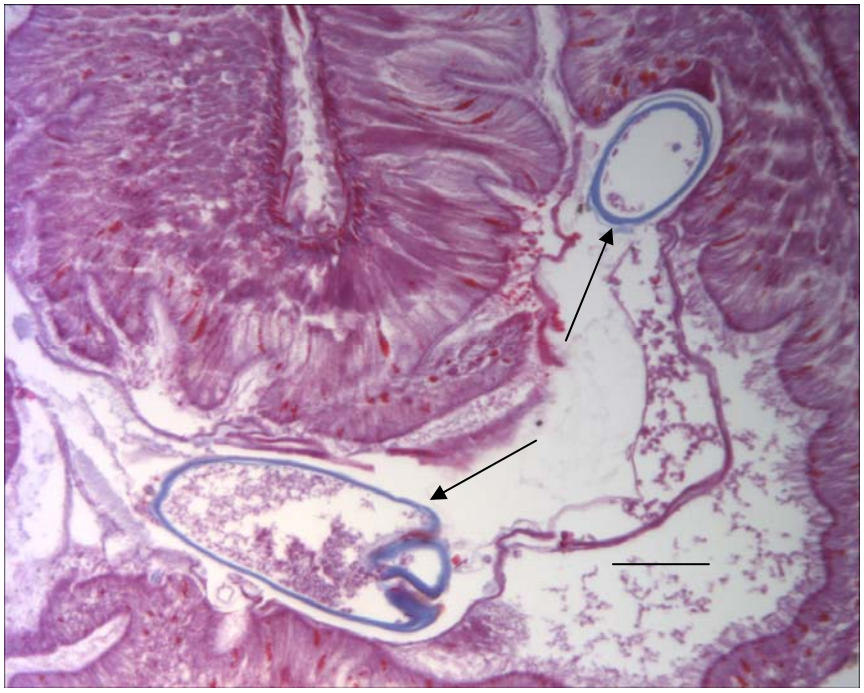


Figure 2.21 Parasaggital section of the digestive tract from fireworm sample 07-55 III. Many materials in the intestine of the specimens were lined with an aniline blue-staining collagenous cuticle (black arrows) encasing purple-staining suspect coccoid bacterial cells. Scale bar = 50 µm

Therefore, the ability to accurately identify the materials in the gut is difficult. However, there are many similarities in the gut contents among the nine specimens in this study. Many of the specimens had numerous aniline blue-staining (Cason's) cuticle-like particles in the lumen. In anterior sections, these materials were intact and encompassed purple coccoid cells (Figure 2.21). In posterior sections, the cuticle-like particles were broken down into strands and the particles were engaged with the epithelial cells of the digestive system. Bits of muscles and adipose tissue were also present with the cuticle materials in the gut of one specimen (Figure 2.22). In other specimens, purple cells (Cason's) resembling bacteria (Figure 2.23) and clusters of chloroplasts were observed.

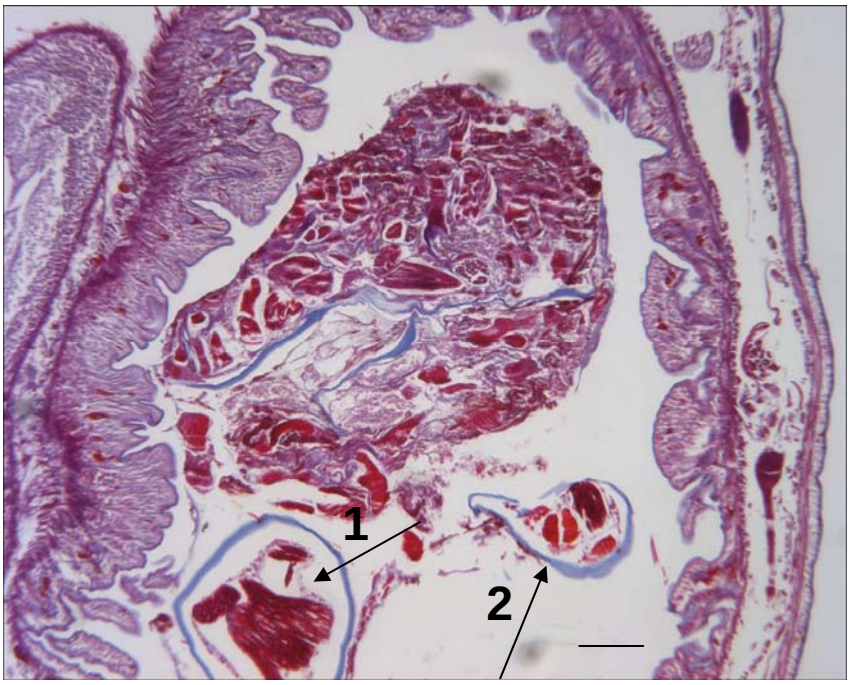


Figure 2.22 Cross-section of the digestive tract from fireworm sample 07-55 IIA. Along with aniline blue-staining cuticles (2), fragments of muscles (1) were also found in the lumens of the fireworms. Scale bar = 100 μm

Reproductive System

This study failed to observe features related to the reproductive system. Marsden (1968) observed *H. carunculata* spawning in the summer. The specimens for these observations were collected during the winter, therefore the gonads may not have been developing due to seasonal conditions. Previous studies have stated the limited understanding of the reproductive system of many polychaetes (Gardiner 1992). *H. carunculata* is one of many species in the class Polychaeta which lacks baseline data on reproductive patterns including gamete production and gonad location. More studies on seasonal spawning and reproductive features of this species are needed to understand

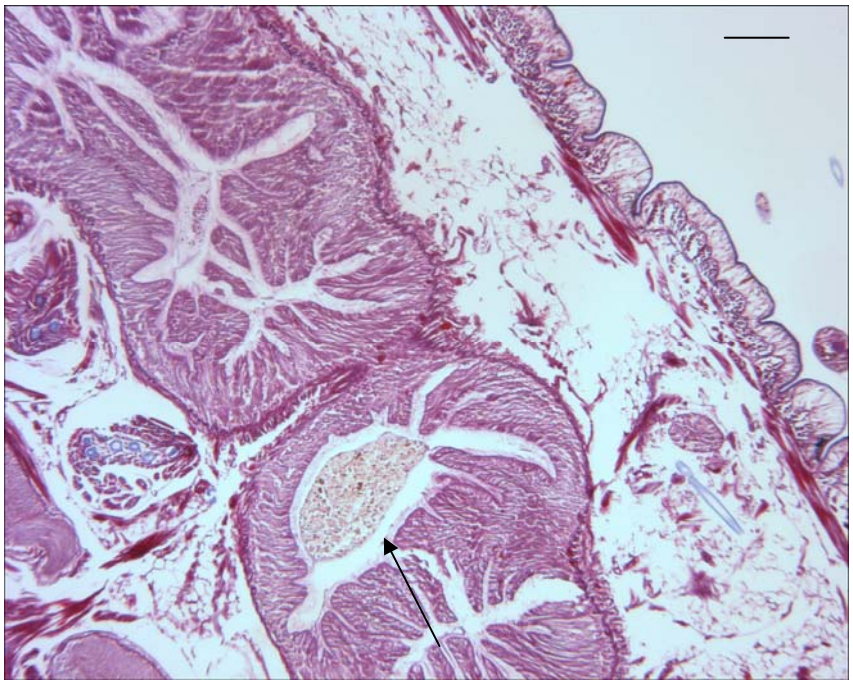


Figure 2.23 Parasaggital section of the digestive tract from fireworm sample 07-53 III. Some specimens had consumed clusters of coccoid cells (black arrow). Some clusters (not pictured) were brown and green in color. Scale bar = 100 µm

the correlation of reproduction to nutritional needs and feeding behaviors.

Discussion

The results confirm some earlier findings about the structure of *H. carunculata* (Marsden 1962, 1965) and the general anatomy of segmented worms (Fauchald 1977, Gardiner 1992). However, some features were not reported previously. More research is needed to understand the implications of these structures on the interaction of *H. carunculata* with its environment.

Integumentary System

The epidermis and outer collagenous cuticle of *H. carunculata* is typical of the Order Amphinomida (Gardiner 1992). Furthermore, the toxic setae in the notopodium and neuropodium have also been reported for *H. carunculata* previously (Marsden 1962). The pigment granules in the epidermis were in different orientations, densities, and variation of colors unique to individual specimens. This histological observation is consistent with field observations of various color schemes for this species (Lewis, unpublished data).

A thin layer of coccoid cells, perhaps bacteria, was found lining the outside of the cuticle on all organisms. There was no evidence of exchange between these cells and the epidermis. This thin layer could be another type of defense mechanism, perhaps a "biofilm." If these cells are microbes, their presence may function to protect the cuticle. Conversely, the cells could feed on the cuticle or the biofilm could pick up microbes known to cause coral disease. Sussman et al. (2003) showed the *Vibrio shilio* cells penetrated the epidermis, but did not mention the existence of a collagenous cuticle nor how the bacteria were able to pass through it. This outer layer of cells has not been previously noted in literature for this species and therefore more research should be done to understand the role and biological nature of this outer cuticle coating.

Circulatory System

The circulatory system consists of various blood vessels including the main blood vessels located dorsally. Blood vessels were also associated with the gut cavity. No new or previously unreported features of the circulatory system were noted in this study.

Nervous System

H. carunculata has multiple features that function to find prey and avoid predators. This species has primitive eyes in the form of two photoreceptor lenses located at the most anterior section of the body. The eyes are connected via nerve fibers to a cerebral ganglion. The cerebral ganglion also receives information from the nuchal organ in the caruncle region located on the dorsal side of the body. The epidermis of the caruncle region is intensely folded and supported on connective tissue, with nerve fibers apparently connecting to epidermal cells, although further study with transmission electron microscopy will be necessary to determine whether the ends of the fibers are modified in some manner to support chemoreception or mechanoreception. A large surface area in this region would increase sensory capability. Therefore, this observation suggests the caruncle region serves to pick up cues from the environment and may serve to locate prey and/or to identify predators.

The most obvious defense mechanism of the species which has been mentioned is previous studies (Marsden 1962) is the toxic setae found in high density in both parapodia. The setae are lined cuticles filled with red granules (H&E stain). Nuclei and other organelles (H&E stain) were not present in these granules. Muscles surround the

base of the setae. The setae appear to grow out of a follicle in the epidermis, which indicates the site of regeneration of broken setae.

An unreported observation of the nervous system is the connectivity of the two-lobed nerve cord to the epidermis. The nerve cord runs ventrally along the body and is connected to the epidermis through a thin connective tissue layer. At the point of contact with the nerve cord, the epidermis has pigmented granules uniquely dense in this area and some cells penetrate the cuticle. Furthermore, the segment in this region is uniquely shaped with a central peak, similar to a keel on a boat, at the site of penetrating granules. The nerve cord may receive information from the external environment via these pigmented cells. This interface outside the body shows it has a sensory function which could be for finding prey as it moves along substrate. An alternative function of these penetrating pigmented granules could also have a mucous function and seep through pores in the cuticle. Marsden (1968) noted a dark mid-ventral line which contained dark material similar to fecal excretions. This region is in the same location as the mid-ventral line and could also serve as another site of waste removal. More observations on this feature are needed to fully understand its function.

Digestive System

The structural features used for consuming prey were examined to understand the ability of *H. carunculata* to forage on various types of organisms. The proboscis is made up of numerous layers of muscles which would aid in extracting materials from a solid substrate. The epithelium of the foregut and the pharynx is lined with a thick cuticle. This

feature may protect the epithelium when exposed to the environment. Though this invertebrate is not equipped with jaws similar to other marine invertebrates (Gardiner 1992), it uses its powerful pharynx muscles for effective predation. Bruckner (2001) documented *H. carunculata* enlarging its pharynx to completely engulf the tips of *A. cervicornis* and removing the coral tissue.

Once food has been consumed, it passes through the foregut and into the digestive tract (Marsden 1963b). The epithelium posterior to the foregut is not lined with a cuticle. Mucocytes and granular secretory cells embedded in the epithelium secrete compounds to work on the materials in the lumen for digestion (Marsden 1966). The materials move through the gut aided by the muscles lining the digestive tract and apical cilia of the thin columnar epithelial cells. The cilia become longer and denser in the posterior section of the gut. Secretory cells and mucocytes become less numerous in the epithelium inverse to the length and density of the cilia. These observations are consistent with previous studies on the digestive tract of this species (Marsden 1966).

Gut content was examined to extrapolate diet composition of this species. Cuticles of various shapes and sizes were found consistently in specimens. In some instances, the cuticles were found with clusters of coccoid cells resembling bacteria. Also, in one specimen, cuticles were found in the same region of the gut as fragmented muscle cells. Clusters of green cells resembling chloroplasts (H&E), brown cells (H&E), and purple cells resembling bacteria were found independent of other materials. The materials in the gut varied which is consistent with previous studies (Marsden 1962, 1963a).

Two features of the foregut, not previously reported, were found in the course of this investigation: clusters of secretory cells in the proboscis region and a specialized tissue within the digestive tract. The orientation of these secretory cells suggests they are released when the pharynx is everted during feeding to breakdown the tissue and aid in consuming materials. However, previous studies have not documented the release of digestive enzymes on the feeding site. This feeding technique, specifically on coral colonies, may induce tissue loss after the predation episode is over. The impact of digestive enzymes on coral tissue needs to be understood to help diagnose causal agents of coral tissue loss in field.

The second new feature is the specialized tissue within the digestive system. It is lined with a cuticle similar to the surrounding epithelium. However, the tissue is not directly attached to the epithelium and comes out into the digestive cavity via a stalk. Its function is unclear and more studies are needed to ascertain the role of this tissue.

Marsden (1963a) identified coral tissue by the presence of nematocysts and clusters of cells that appeared to be zooxanthellae. However, her findings were not coordinated with active coral feeding observations. The specimens collected for this study were also not actively feeding on coral and evidence of coral tissue, including the presence of nematocysts, based on Marsden's criteria, was missing. *H. carunculata* can take up to 9 hours to fully digest materials (Marsden 1968). Nevertheless, these corallivores did not have evidence of coral tissue in their gut which would indicate these specimens were feeding on alternative prey in the presence of coral tissue. Due to the lack of documented studies on identifying coral materials in gut contents, this study can

not draw conclusions about the presence or absence of coral tissue in the digestive tract. Future studies are needed to coordinate feeding observationswith histological and physiological analysis to obtain a more complete picture of the appearance of coral tissue in the gut and document the passage of coral through the digestive system.

H. carunculata has not been researched extensively. To date, most studies have focused on the digestive tract (Marsden 1962, 1963), on foraging cycles (Lewis and Crooks 1996), or on field observations at one site (Miller and Williams 2007). Sussman et al. (2003) found this species to be a vector for the bleaching pathogen *V. shioli* but did not provide information on the length of time the bacteria were retained in the specimens' bodies. Despite these studies, baseline information on diet, feeding preferences, and trophodynamics is lacking for this species. The results from this study indicate this species has features, like a "biofilm," a ventral sensory feature, and secretory cells in the proboscis, which may be key elements in its interactions with its prey and benthic habitat. Also, the observations, at least from this sample of worms, indicate coral tissue is not a vital part of this species' diet. Since detailed information on *H. carunculata*'s diet composition is lacking, it is difficult to determine the impact of predation either on coral or on its alternative prey. The presence of secretory cells in the proboscis suggests this species may use digestive enzymes to aid in consumption of food. Furthermore, the pharynx has tremendous elasticity to enlarge and is supported by layers of muscles to consume prey. These features will aid *H. carunculata* to adapt its feeding behavior to consume different, more abundant, types of prey.

As available coral tissue declines worldwide, the loss of coral reef biological and physical structures are impacting the survivorship of coral reef inhabitants. More research is needed to better understand *H. carunculata*'s role in coral reef ecosystems to predict the impact of coral reef decline on this species and its prey. Studies on the adaptability of these coral reef inhabitants are needed to understand their ability to survive in a changing environment.

Literature Cited

Adams AJ, Ebersole JP (2002) Use of back-reef and lagoon habitats by coral reef fishes. Mar Ecol-Prog Ser 228:213-226

Alvarez-Filip L, Gil I (2006) Effects of Hurricanes Emily and Wilma on coral reefs in Cozumel, Mexico. Coral Reefs 25:583-583

Aronson RB, MacIntyre IG, Wapnick CM, O'Neill MW (2004) Phase shifts, alternative states, and the unprecedented convergence of two reef systems. Ecology 85:1876-1891

Baums IB, Miller MW, Szmant AM (2003a) Ecology of a corallivorous gastropod, *Coralliophila abbreviata*, on two scleractinian hosts. 1: Population structure of snails and corals. Mar Biol 142:1083-1091

Baums IB, Miller MW, Szmant AM (2003b) Ecology of a corallivorous gastropod, *Coralliophila abbreviata*, on two scleractinian hosts. II. Feeding, respiration and growth. Mar Biol 142:1093-1101

Bell JD, Galzin R (1984) Influence of live coral cover on coral-reef fish communities. Mar Ecol-Prog Ser 15:265-274

Bellwood DR, Hoey AS, Ackerman JL, Depczynski M (2006) Coral bleaching, reef fish community phase shifts and the resilience of coral reefs. Glob Change Biol 12:1587-1594

Berumen ML, Pratchett MS, McCormick MI (2005) Within-reef differences in diet and body condition of coral-feeding butterflyfishes (Chaetodontidae). Mar Ecol-Prog Ser 287:217-227

Booth DJ, Wellington G (1998) Settlement preferences in coral-reef fishes: Effects on patterns of adult and juvenile distributions, individual fitness and population structure. Blackwell Science, p 274-279

Bruckner AW (2001) Coral health and mortality. Recognizing the signs of coral diseases and predators. In: Humann P, Deloach N (eds) Reef Coral Identification: Florida, Caribbean, Bahamas, Including Marine Plants, p 240-271

Cabaitan PC, Gomez ED, Alino PM (2008) Effects of coral transplantation and giant clam restocking on the structure of fish communities on degraded patch reefs. J Exp Mar Biol Ecol 357:85-98

Carr M, Hixon M (1997) Artificial reefs: the importance of comparisons with natural reefs. Mar Ecol-Prog Ser 22:28-33

Cheal A, Wilson SK, Emslie MJ, Dolman A, Sweatman H (2008) Responses of reef fish sommunities to coral declines on the Great Barrier Reef. Mar Ecol-Prog Ser 372:211-223

Connell SD, Kingsford MJ (1998) Spatial, temporal and habitat related variation in the abundance of large predatory fish at One Tree Reef, Australia. Coral Reefs 17:49-57

Done TJ (1992) Phase shifts in coral reef communities and their ecological significance Hydrobiologia 247:121-132

Fauchald K (1977) The Polychaete Worms: Definitions and Keys to the Orders, Families and Genera, Vol 28. Natural History Museum of Los Angeles County, Los Angeles

Gardiner S (1992) Annelida. In: Harrison F (ed) Microscopic Anatomy of Invertebrates, Vol 7. Wiley-Liss, New York

Hayes JA (1990) Prey preference in a Caribbean corallivore, *Coralliophila abbreviata* (Lamark) (Gastropoda, Coralliophilidae). . Bull Mar Sci 47:557-560

Hoegh-Guldberg O, Mumby PJ, Hooten AJ, Steneck RS, Greenfield P, Gomez E, Harvell CD, Sale PF, Edwards AJ, Caldeira K, Knowlton N, Eakin CM, Iglesias-Prieto R, Muthiga N, Bradbury RH, Dubi A, Hatziolos ME (2007) Coral reefs under rapid climate change and ocean acidification. Science 318:1737-1742

Hughes TP, Rodrigues MJ, Bellwood DR, Ceccarelli D, Hoegh-Guldberg O, McCook L, Moltschaniwskyj N, Pratchett MS, Steneck RS, Willis B (2007) Phase shifts, herbivory, and the resilience of coral reefs to climate change. Curr Biol 17:360-365

Hughes TP, Tanner JE (2000) Recruitment failure, life histories, and long-term decline of Caribbean corals. Ecology 81:2250-2263

Jones GP, McCormick MI, Srinivasan M, Eagle JV (2004) Coral decline threatens fish biodiversity in marine reserves. Proc Natl Acad Sci U S A 101:8251-8253

Kokita T, Nakazono A (2001) Rapid response of an obligately corallivorous filefish *Oxymonacanthas longirostris* (Monacanthidae) to a mass coral bleaching event. Coral Reefs 20:155-158

Lewis JB, Crooks RE (1996) Foraging cycles of the amphinomid polychaete *Hermodice carunculata* preying on the calcareous hydrozoan Millepora complanata. Bull Mar Sci 58:853-856

Lindahl U, Ohman MC, Schelten CK (2001) The 1997/1998 mass mortality of corals: Effects on fish communities on a Tanzanian coral reef. Mar Pollut Bull 42:127-131

Lizama J, Blanquet RS (1975) Predation on sea anemones by the amphinomid polychaete, *Hermodice carunculata*. Bull Mar Sci 25:442-443

Marsden JR (1962) A coral-eating polyachete. Nature 193:598

Marsden JR (1963a) A preliminary report on digestive enzymes of *Hermodice carunculata*. Canadian Journal of Zoology/Revue Canadien de Zoologie 41:159-164

Marsden JR (1963b) The digestive tract of *Hermodice carunculata.* (Pallas). Polychaeta: Amphinomidae. Canadian Journal of Zoology/Revue Canadien de Zoologie 41:165-184

Marsden JR (1966) The coelomocytes of *Hermodice carunculata* (Polychaeta: Amphimomidae) in relation to digestion and excretion. Canadian Journal of Zoology/Revue Canadien de Zoologie 44:377-389

Martin A, Losada F (1991) Regeneration of Injuries caused by the Amphinomid polychaete *Hermodice carunculata* on scleractinian corals, hydrocorals and octocorals (Cnidaria). Ecotropicos 4:18-26

Menge BA, Lubchenco J (1981) Community organization in temperate and tropical rocky intertidal habitats - prey refuges in relation to consumer pressure gradients Ecol Monogr 51:429-450

Miller MW, Williams DE (2007) Coral disease outbreak at Navassa, a remote Caribbean island. Coral Reefs 26:97-101

Motta PJ (1988) Functional morphology of the feeding apparatus of 10 species of pacific butterflyfishes (Perciformes, Chaetodontidae) – an ecomorphological approach. Environ Biol Fishes 22:39-67

Mumby PJ, Dahlgren CP, Harborne AR, Kappel CV, Micheli F, Brumbaugh DR, Holmes KE, Mendes JM, Broad K, Sanchirico JN, Buch K, Box S, Stoffle RW, Gill AB (2006) Fishing, trophic cascades, and the process of grazing on coral reefs. Science 311:98-101

Newman MJH, Paredes GA, Sala E, Jackson JBC (2006) Structure of Caribbean coral reef communities across a large gradient of fish biomass. Ecol Lett 9:1216-1227

Ott B, Lewis JB (1972) The importance of the gastropod *Coralliophila abbreviata* (Lamarck) and the polychaete *Hermodice carunculata* (Pallas) as coral reef predators. Can J Zool/Rev Can Zool 50:1651-1656

Pandolfi JM, Jackson JBC (2006) Ecological persistence interrupted in Caribbean coral reefs. Ecol Lett 9:818-826

Pandolfi JM, Jackson JBC, Baron N, Bradbury RH, Guzman HM, Hughes TP, Kappel CV, Micheli F, Ogden JC, Possingham HP, Sala E (2005) Ecology - Are US coral reefs on the slippery slope to slime? Science 307:1725-1726

Pratchett MS, Munday PL, Wilson SK, Graham NAJ, Cinner JE, Bellwood DR, Jones GP, Polunin NVC, McClanahan TR (2008) Effects of climate-induced coral bleaching on coral-reef fishes - Ecological and economic consequences. In: Oceanography and Marine Biology: an Annual Review, Vol 46, Vol 46. Crc Press-Taylor and Francis Group, Boca Raton, p 251-296

Pratchett MS, Wilson SK, Baird AH (2006) Long-term monitoring of the Great Barrier Reef. J Fish Biol 69:1269-1280

Pratchett MS, Wilson SK, Berumen ML, McCormick MI (2004) "Sublethal effects of coral bleaching on an obligate coral feeding butterflyfish". Coral Reefs 23:352-356

Rotjan RD, Lewis SM (2005) Selective predation by parrotfishes on the reef coral Porites astreoides. Mar Ecol-Prog Ser 305:193-201

Rotjan RD, Lewis SM (2008) Impact of coral predators on tropical reefs. Mar Ecol-Prog Ser 367:73-91

Samways MJ (2005) Breakdown of butterflyfish (Chaetodontidae) territories associated with the onset of a mass coral bleaching event. Aquatic Conservation: Marine and Freshwater Ecosystems 15:S101-S107

Sano M, Shimizu M, Nose Y (1984) Changes in structure of coral-reef fish communities by destruction of hermatypic corals - observational and experimental views Pacific Science 38:51-79

Smith JE, Shaw M, Edwards RA, Obura D, Pantos O, Sala E, Sandin SA, Smriga S, Hatay M, Rohwer FL (2006) Indirect effects of algae on coral: algae-mediated, microbe-induced coral mortality. Ecol Lett 9:835-845

Smith TB, Nemeth RS, Blondeau J, Calnan JM, Kadison E, Herzlieb S (2008) Assessing coral reef health across onshore to offshore stress gradients in the US Virgin Islands. Mar Pollut Bull 56:1983-1991

Sussman M, Loya Y, Fine M, Rosenberg E (2003) The marine fireworm *Hermodice carunculata* is a winter reservoir and spring-summer vector for the coral-bleaching pathogen Vibrio shiloi. Environmental Microbiology, 2003 Apr, 5(4):250-5

Syms C, Jones GP (2000) Disturbance, habitat structure, and the dynamics of a coral-reef fish community. Ecology 81:2714-2729

Tricas TC (1989) Determinants of feeding territory size in the corallivorous butterflysih, *Chaetodon multicinctus* Anim Behav 37:830-841

Wilkinson C (ed) (2004) Status of coral reefs of the world, Vol 1. Global Coral Reef Monitoring Network and Australian Institute of Marine Science, Townesville, Australia

Wilson SK, Graham NAJ, Pratchett MS, Jones GP, Polunin NVC (2006) Multiple disturbances and the global degradation of coral reefs: are reef fishes at risk or resilient? Glob Change Biol 12:2220-2234

Witman JD (1988) Effects of predation by the fireworm *Hermodice carunculata* on Milleporid hydrocorals. Bull Mar Sci 42:446-458

Yap HT (2004) Differential survival of coral transplants on various substrates under elevated water temperatures. Mar Pollut Bull 49:306-312

Yap HT (2009) Local changes in community diversity after coral transplantation. Mar Ecol-Prog Ser 374:33-41

3. The effect of a *Millepora complanata* feeding regime on the body weight of *Hermodice carunculata*

Abstract

As coral reef species densities and distributions shift in response to environmental changes, impacts on benthic coral reef communities need to be well understood. Dietary habitats and preferences are important considerations when determining the consequences of coral reef decline on corallivores and their prey. However, the effects of decreased available coral tissue on the nutrient intake of direct coral consumers, like the invertebrate corallivore *Hermodice carunculata,* has not been addressed extensively in previous studies. *H. carunculata* is commonly found on tropical reefs including off Barbados. Barbados reefs have experienced high percentage of coral colony mortality over the past 30 years. The hydrocoral *Millepora complanata* is now found in higher densities than previously abundant coral species like *Montastraea annularis*. *Ex situ* experiments were conducted to examine the weight change of *H. carunculata* organisms under a feeding regime of *M. complanata* as compared with a feeding regime of a previously abundant species *M. annularis*. This study showed a *M. complanata* feeding regime did not have a substatantal impact on the body weight of *H. carunculata* over time.

Introduction

Coral populations are declining worldwide (Knowlton 2001, Gardner et al. 2003, Hughes et al. 2003, Pandolfi et al. 2003, Bellwood et al. 2004). The causes have been attributed to various factors both human-induced and biotic, including overfishing (Jackson et al. 2001, Myers and Worm 2003), diseases (Pandolfi et al. 2003, Sutherland et al. 2004), storms (Bythell et al. 1993, Hughes 1994, Knowlton 1992, Alvarez-Filip and Gil 2006), and elevated sea temperatures (Runnalls and Coleman 2003, Edmunds 2004, Rotjan and Lewis 2008). Coral mortality has been particularly widespread throughout the Caribbean (Connell 1997, Green et al. 2008, Mumby et al. 2007, Pandolfi et al. 2003). It has been estimated that Caribbean corals have declined by 80% in the last 30 years (Richardson and Voss 2005). Coral reefs off of Barbados have undergone disturbances similar to the wider Caribbean region (Runnalls and Coleman 2003, Macintyre et al. 2007). Coral cover has decreased and once dominate coral species, like *Acropora palmata*, have disappeared (Aronson and Precht 2001, Aronson et al. 2004, Macintyre et al. 2007). In place of the previously abundant scleractinian corals like *Montastraea* and *Acropora*, species with high fecundity like *Porites* (Chornesky and Peters 1987, McGuire 1998) have become dominant in the community structure through increase in percentage cover (Green et al. 2008).

Mass mortality and phase shift of coral species can have implications for reef biodiversity (May 1977, Chapin et al. 2000, Lokrantz et al. 2008) and specifically impact marine species which rely on coral reefs for all or part of their life cycle (Bellwood et al. 2003, Folke et al. 2004). While short-term and long-term factors continue to lead to coral

loss (Richardson and Voss 2005, Mumby et al. 2007), coral reef management plans must evaluate the impact of newly dominant coral species on community ecology (Chapin et al. 2000, Pandofli et al. 2003, Lokrantz et al. 2008). More specifically, the decline of coral populations can have an important impact on direct consumers of coral tissue, corallivores (Baums et al. 2003a, Rotjan and Lewis 2008).

The role of corallivores in reef communities is important and complex (Glynn 2004, Knowlton 1992, Rotjan et al. 2006, Rotjan and Lewis 2008). Coral predators total more than 160 known species representing 11 families of fishes (Motta 1988) and 5 invertebrate phyla (Dalton and Godwin 2006, Bak and Steward-Van Es 1980, Lester and Bak 1985, Oren et al. 1997). Corallivores practice two types of feeding mode: obligate feeders, which eat exclusively live coral, and facultative feeders, which eat mixed diets including coral. Corallivores use different feeding strategies which have varying levels of impact on coral prey (Rotjan and Lewis 2008). There are four categories used to classify the feeding techniques of most corallivores: browsers, excavators, mucus-feeders, and scrapers.

'Browsers' generally remove live coral tissue without removing the underlying skeleton (Hiatt and Strasburg 1960). 'Excavators' and 'scrapers' remove both tissue and skeleton (Bellwood and Choat 1990). However, 'excavators' remove a considerable amount of skeleton while 'scrapers' only take a small portion of the skeleton with the attached tissue. 'Mucus-feeders' consume the coral mucus without removing tissue and/or skeleton (Rotjan and Lewis 2008).

Corallivores can be found in the vertebrate and invertebrate groups. There are reportedly 114 species of vertebrate corallivores (Rotjan and Lewis 2008). About half the total corallivorous vertebrates are butterfly fishes and exhibit all four feeding strategies described above (Motta 1988). The other piscine corallivores are either scrapers or excavators and include damselfish, filefish, triggerfish, and wrasses (Bellwood and Choat 1990). *Acropora, Montipora, Pocillopora,* and *Porites* are the corals most commonly grazed on by these fishes (Rotjan and Lewis 2008).

Annelids, anthropods, echinoderms, and mollusks make up the 51 species of invertebrate corallivores (Rotjan and Lewis 2008). All four feeding strategies are used by at least one species. Due to their rate of tissue consumption (Moran 1986, Colgan 1987, Turner 1994) and periodic population outbreaks (Birkeland and Lucas 1990, Glynn 1990, Turner 1994, Houk et al. 2007), two influential invertebrate corallivores are *Acanthaster planci* (145 cm^2 d^{-1}) (Morton et al. 2002) and *Drupella* spp. (137-229 bites m^{-1}) (Rotjan and Lewis 2008). Localized surges in their population densities can result in rapid loss of coral tissue (Rotjan and Lewis 2008) and reduced coral survival (Colgan 1987).

Food preferences for invertebrate corallivores vary but mostly consist of *Acropora, Millepora, Montipora, Pocillopora,* and *Porites* (Morton et al. 2002). Diet has been shown to have a major influence on the population structure of one invertebrate corallivore, the corallivorous gastropod, *Coralliophila abbreviata* (Baums et al. 2003a). Snails feeding on *A. palmata* grew faster than snails residing on *M. annularis* colonies even though the tissue of *M. annularis* provides more nitrogen (N) and carbon (C) per unit area than *A. palmata* (Baums et al. 2003b). Baums et. al (2003b) found feeding rates

depended on the nutritional value of the coral prey (*M. annularis*: 10.9±0.5 C:N ratio; 0.13-0.88 cm^2 day^{-1}; *A. palmata*: 6.3±0.3 C:N ratio; 0.44-3.28 cm^2 day^{-1}).

Population aggregations and parameters of invertebrate corallivores can be affected by coral species diversity and abundance (Ott and Lewis 1972, Glynn 1990, Hayes 1990, Baums et al. 2003a). However, more studies are needed to understand invertebrate corallivory feeding preference and consequential tissue loss impact (Martin and Losada 1991, Baums et al. 2003b, Williams and Miller 2005, Rotjan and Lewis 2008).

Hermodice carunculata is an invertebrate corallivore which has not been studied extensively despite recognition of its important role in coral mortality (Ott and Lewis 1972, Knowlton 1992, Lewis and Crooks 1996) and etiology of coral disease (Sussman et al. 2003, Miller and Williams 2007). *H. carunculata* is a segmented polychaete from the Family Amphinomidae. Its dorsal parapodia contain toxic setae which are used as a defense mechanism (Marsden 1963). It is widely distributed in the tropical and subtropical areas of the Atlantic Ocean, the Southern Atlantic Ocean, and the Mediterranean Sea (Martin and Losada 1991). *H. carunculata* is a facultative browser (Rotjan and Lewis 2008) and is known to consume *Acropora, Millepora, Montastraea, Oculina,* and *Porites* coral species (Glynn 1963, Ott and Lewis 1972, Witman 1988) along with sponges and other invertebrates (Marsden 1963, Lizama and Blanquet 1975).

The majority of the few studies conducted on this species have focused on the Barbados populations of *H. carunculata* and their predation on the hydrozoan fire corals of the genus *Millepora.* Witman (1988) calculated *H. carunculata* consumption rate of

Millepora to be 12.9cm^2 d^{-1}. A separate study (Martin and Losada 1991) found the tissue regeneration percentage and rate for *Millepora* was 44% and 0.06 cm^2/day, respectively, after predation by *H. carunculata*. Lewis and Crooks (1996) monitored a large *Millepora* colony to determine the foraging cycle of *H. carunculata* was mostly during the late afternoon.

Similar to *Porites*' ability to dominate disturbed systems, fire corals have been successful and spatially dominant in shallow coral reef systems (de Weerdt 1981, Witman 1992, Lewis 2006), locally abundant and important reef-framework builders (Lewis 1989, Edmunds 1999). As rapid growers and with the use of both asexual and sexual reproduction, they are often the first to recover from local disturbances like bleaching (Lewis 2006). There are some general similarities between these hydrocorals and scleractinian corals, such as having calcareous skeletons and symbiotic dinoflagellates (Sheppard 1982, Lewis 1992). However, fire corals differ from scleractinians in their morphological strategy (Edmunds 1999) and energy consumption (Lewis 1992). They can grow thin encrusting bases which can spread across substrate and produce dense branches (Wahle 1980, Witman 1992). One benefit to this structural characteristic is the ability to survive wave stress (Lewis 1989). Despite fire corals protruding polyps which contain highly toxic nematocysts (Lewis 2006), a variety of predators and burrowing animals rely on this hydrocoral (Lewis 2006), and, hence, fire corals are also important to the community ecology of reef ecosystems (Edmunds 1999).

M. complanata and *M. alcicornis* can be found in large densities in the Caribbean (Edmunds et al. 1990). In contrast, *M. annularis* has become reduced in density on most

Caribbean reefs (Tomascik and Sander 1987, Hughes and Tanner 2000, Edmunds and Elahi 2007). Since *Millepora* is abundant in the shallow reef systems while *M. annularis* has experienced a decrease in density, this project addressed the impact of increased *M. complanata* spatial dominance on the body weight of the corallivore *H. carunculata* through *ex situ* experiments. The physiological effect of a *Millepora* feeding regime on the body weight of *H. carunculata* was compared to the body weight of *H. carunculata* under a *M. annularis* feeding regime.

Field observations in Barbados indicate *H. carunculata* feeds on fire coral frequently (Ott and Lewis 1972, Witman 1988, Lewis and Crooks 1996). Thus, this observed feeding behavior would indicate that *M. complanata* is a substantial food source and *H. carunculata* body weight will be greater in the presence of a *M. complanata* feeding regime than when feeding on *M. annularis.* The working hypothesis is if *H. carunculata* feeds on fire coral (*M. complanata)*, its body weight will be greater than its body weight when it has been feeding on stony corals. The null hypothesis for this study is if *H. carunculata* feeds on fire coral (*M. complanata)*, its body weight will not be greater than its body weight when it has been feeding on stony corals.

Methods

The effect of a *M. complanata* feeding regime on the body weight of *H. carunculata* was tested through *ex situ* experiments in the laboratories of the Bellairs Research Institute in Barbados in November and December 2005 and February–April 2006. The specimens were randomly selected from the population of *H. carunculata* on

the west coast shallow fringing reefs primarily Batts Rock Reef which had high densities of *M. complanata* (S. Lewis, unpublished data). The wet weight of the specimens were measured at several intervals and compared with the wet weight of specimens experiencing a shift in feeding regimes to *M. annularis*.

Experiment Set-up:

The laboratory feeding experiments were conducted in five custom-made glass aquaria (4 x 2 x 3 ft) using a flow-through seawater system in the wet laboratory at the Bellairs Research Institute in Barbados. Prior to the start of the experiments, the aquaria were filled with water to leach any toxins from the adhesives used on the tanks. After one week, the tanks were emptied and scrubbed clean. The flow-through seawater system ran for three days to further flush out the tanks. Prior to the start of the experiments, sand was gathered from the nearshore beach and sifted through a #3 mesh screen. The sifted sand was then placed in the bottom of each tank to create an 8 cm-thick layer.

To limit the food resources of the specimens to only the coral colonies provided in the tank (i.e., reduce availability of phytoplankton and zooplankton, larvae, bacteria, etc.), the tanks were scrubbed clean and the water was drained to 10 cm above the sand and refilled completely with the flow-through seawater system each week. During the experiments, the flow-through seawater system ran for 8 hours each day for 4 consecutive days per week. A large aerator was placed in each tank and ran continuously.

Measurements:

The body weight was determined by measuring wet weight on a Fisher Scientific digital bench scale. To prevent physical stress to the specimens, seawater from one of the five aquaria was poured into the scale tray to a depth appropriate for the specimens to be completely immersed (around 4cm). The scale was tared with the water present. Each specimen was placed in the tray and the weight recorded. After the weight was recorded and video images were captured, the specimen was returned to the designated aquarium and the next specimen was measured. All statistical analyses of the results were done using SPSS17.0 software program. The tests were evaluated within a 90% confidence interval ($p \leq 0.10$ is statistically significant).

Experiments:

Two experiments were performed with this equipment and general set-up. The first experiment (1FCE) ran for twenty-six days and all specimens were exposed to a feeding regime of *M. complanata* the entire length of the experiment. For 1FCE, each tank was divided into three sections using mesh screens attached to PVC frames immersed in the tanks. Each section housed an individual coral colony (15 sections of a *M. complanata* sample in total) and a *H. carunculata* specimen (n =15), which were collected at random from nearshore reefs. The wet weight of each worm was measured six times throughout the experiment (days 0, 3, 5, 11, 13, and 26). On day 11, the colonies were removed and replaced with new *M. complanata* colonies on day 13. Thus,

the worms experienced a starving period for three days. After the completion of 1FCE, the worms were removed from the tanks.

In the second experiment (2FCE), the mesh screens were removed from the tanks. The 2FCE ran for 109 days and involved two feeding regimes at different times. For a total of 94 days, 13 specimens (two escaped from the tanks during the experiment) were provided with *M. complanata* colonies. The first round of colonies was removed on day 79 and the worms experienced starvation for the next 6 days. New *M. complanata* colonies were introduced into the tanks on day 87. The worms continued to feed on *M. complanata* colonies until day 94 when these fire coral colonies were immediately replaced with *M. annularis* colonies. The experiment with the new feeding regime of *M. annularis* (2FCE M. ann) ran for 15 days. The wet weight of each worm was measured nine times throughout the experiment (days 0, 11, 28, 53, 79, 87, 94, 103, and 109).

Results:

Analysis of overall weight change for each experiment (1FCE, 2FCE, 2FCE M. ann)

The initial average weight of the specimens in 1FCE (n = 15) was 1.07 g ± 0.502. The end average weight of specimens in 1FCE (n = 15) was 0.947 g ± 0.407. Although the average weight decreased during this time, a Mann-Whitney U test showed this difference was not significant: U = 100.5; exact p = 0.624.

The initial average weight measurements for the 2FCE specimens (n=14) was 1.61 g±0.548. At the end of the *Millepora complanata* feeding regime, the average weight of specimens in 2FCE (n=12) was 1.36 g±0.511. Similar to 1FCE, the mean

weight for the specimens decreased. A Mann-Whitney U test showed this difference was also not insignificant: U=48.0; exact p=0.178.

These end weights for 2FCE are also the initial measurements of the 2FCE specimens at the beginning of the *M. annularis* feeding regime (2FCE M. ann). After 15 days under the new feeding regime, the average weight of specimens (n=12) in 2FCE was 1.13 g±0.551. Compared with the average weight 1.36 g±0.511, the specimens' weight decreased. However, a Mann-Whitney U test showed this difference was not significant: U=61.5; p=0.551.

Even though the average weight decreased in all three experiments, none of the differences were significant. To examine the change in body weight over time during the experiment, the average weight change for each group after each measurement was calculated to analyze the difference between the means (Mann-Whitney U test) and the covariance (Pearson's correlation) of body weight over time between groups (1FCE, 2FCE, 2FCE M. ann).

Analysis of change in weight over time for the Millepora complanata experiments (1FCE, 2FCE)

The trend in body weight was determined from the average change in group weight between measurement intervals (5 in 1FCE and 8 in 2FCE). At each measurement interval, the last weight of each worm was subtracted from the new weight. The average weight change was calculated across each group (1FCE, 2FCE, 2FCE M. ann). The

weight change data for each interval from 1FCE and 2FCE were individually plotted along a line graph and scatterplot to examine the trend line (Figure 3.1).

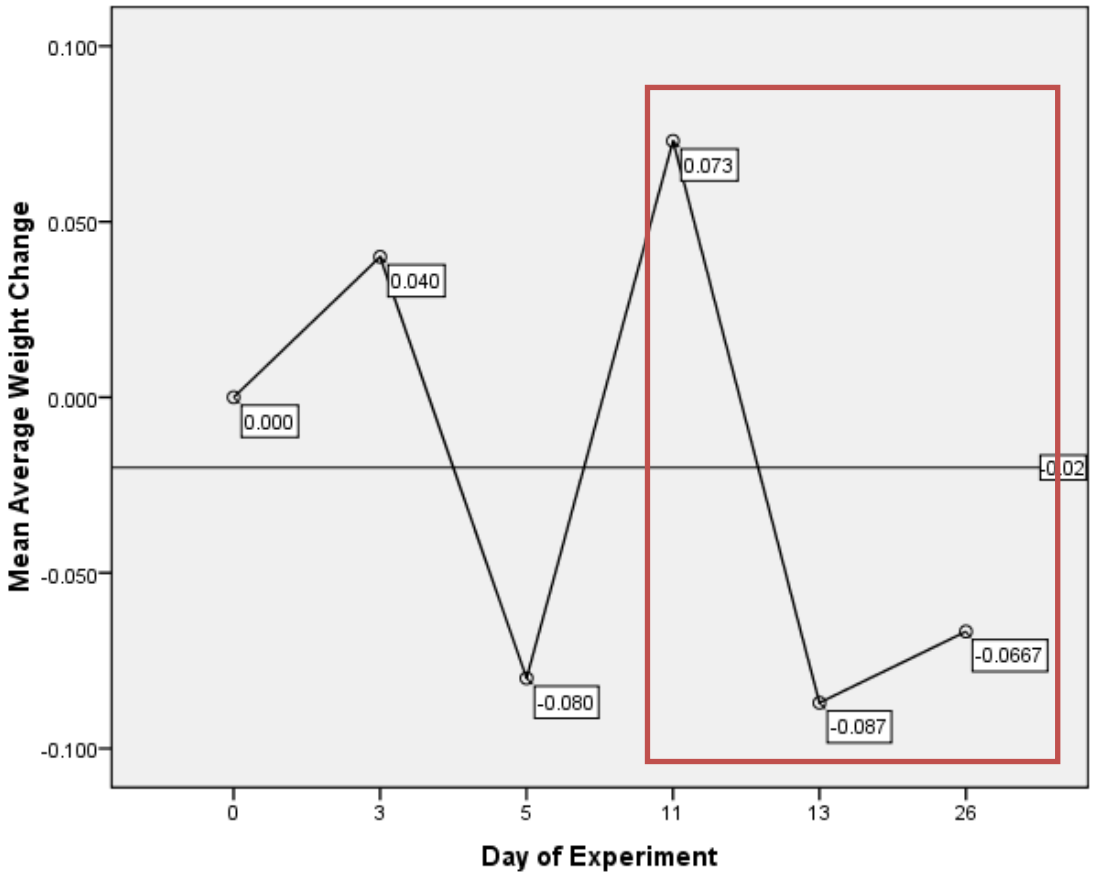


Figure 3.1 The graph shows the average weight change (grams) for the specimens in 1FCE over the duration of the experiment (26 days). The horizontal line in the graph indicates the average weight change for each interval (-0.02 grams). The box highlights the data points used for the comparison of weight change in the absence and presence of *Millepora complanata*.

The average weight change during 1FCE was -0.02. The slope of the best fit line was -0.003. This indicates there was a negative trend in the experiment. On average, the

worms in 1FCE lost weight during the experiment however, there were oscillations in positive and negative weight changes. The greatest decrease in weight occurred between days 11 and 13 which encompassed the starvation time period. This comparison is tested for correlation and significance in the next section.

The slope in the 2FCE graph was also negative. The mean average weight change was -0.04 g (Figure 3.2) which is a slightly larger decrease in weight than 1FCE. The slope of the line was -0.0008. As in 1FCE, the greatest increase and decrease in weight occurs during a period of food removal and reintroduction.

The average weight change for 2FCE M. ann was -0.08 g (Figure 3.3). When the data points were graphed linearly, the slope was 0.007. The three weight change measurements for this experiment were: day 94= -0.108 g; day 103=-0.15; and day 109=0.017. Therefore, only one time interval was positive. A pattern of negative growth was evident in all three data sets. In order to test whether the patterns were significant, difference in the means and the correlation between the data sets were analyzed.

Comparison of covariance and difference in the means between Millepora complanata experiments

The average weight change data sets from 1FCE and 2FCE were compared for correlation (Pearson's correlation) and difference in the means (t-test). The first statistical test was run with the full data sets, including the two data points from a different food regime in 2FCE.

The Pearson's correlation r-value was 0.006 with a p-value of 0.992. This indicates the correlation is not significant between 1FCE and 2FCE. The Levene's test

(F) for equal variance was 2.949; p=0.11. Therefore equal variance is not assumed and the nonparametric equivalent test, Mann-Whitney U, was used. A Mann-Whitney U test showed the difference between the means of 1FCE and 2FCE (full set) was not significant: U=19.50; p=0.388.

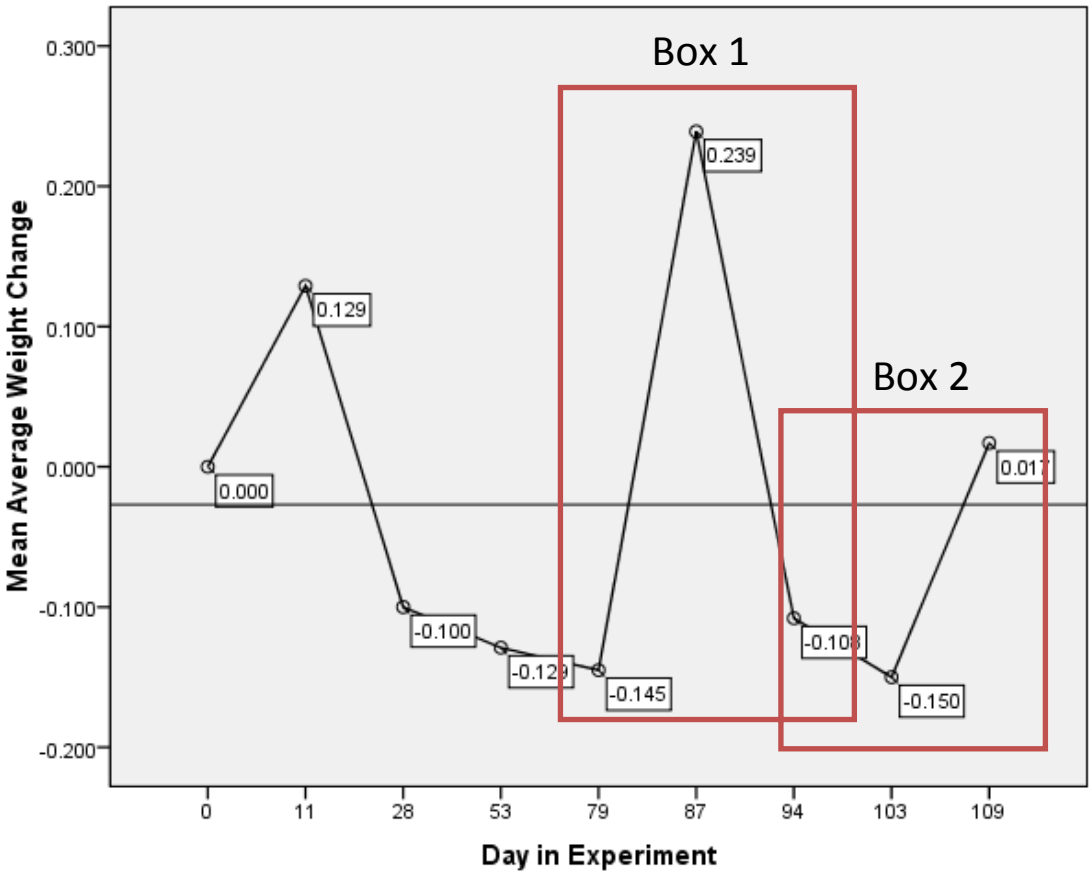


Figure 3.2 The graph shows the average weight change (grams) for the specimens in 2FCE (all) over the duration of the experiment (109 days). The horizontal line in the graph indicates the mean weight change (-0.03 grams). Box 1 highlights the data points used for the comparison of weight change in the absence and presence of *Millepora complanata*. Box 2 highlights the data points used for the comparison of weight change after the switch in feeding regimes to *Montastraea annularis.*

The second statistical test was with 1FCE and 2FCE without the M. ann data points (day 103 and day 109). The Levene's test (F) for equal variance was 3.837; p=0.08. The equal variance is assumed and the parametric t-test can be used to determine the association between the means. The t-value was -0.168 with a p-value of 0.875. Although there was a negative correlation, the association was not statistically significant. The Pearson's correlation value for this association was also not significant (r=-0.665; p=0.537).

Comparison of body weight change between Millepora complanata removal and reintroduction

At one point during experiments 1FCE (Box in Figure 3.1) and 2FCE (Box 1 in Figure 3.2), the *M. complanata* colonies were removed from the tanks for several days before new *M. complanata* colonies were reintroduced. Pearson's correlation (r) was used to compare the linear relationship between three variables (average weight with food (WWF), weight without food (WOF), and weight after introduction of new food (WNF)). Since the Levene's test was not significant for these data sets, a Mann-Whitney U test was used to calculate the significance of the difference in means between the treatments.

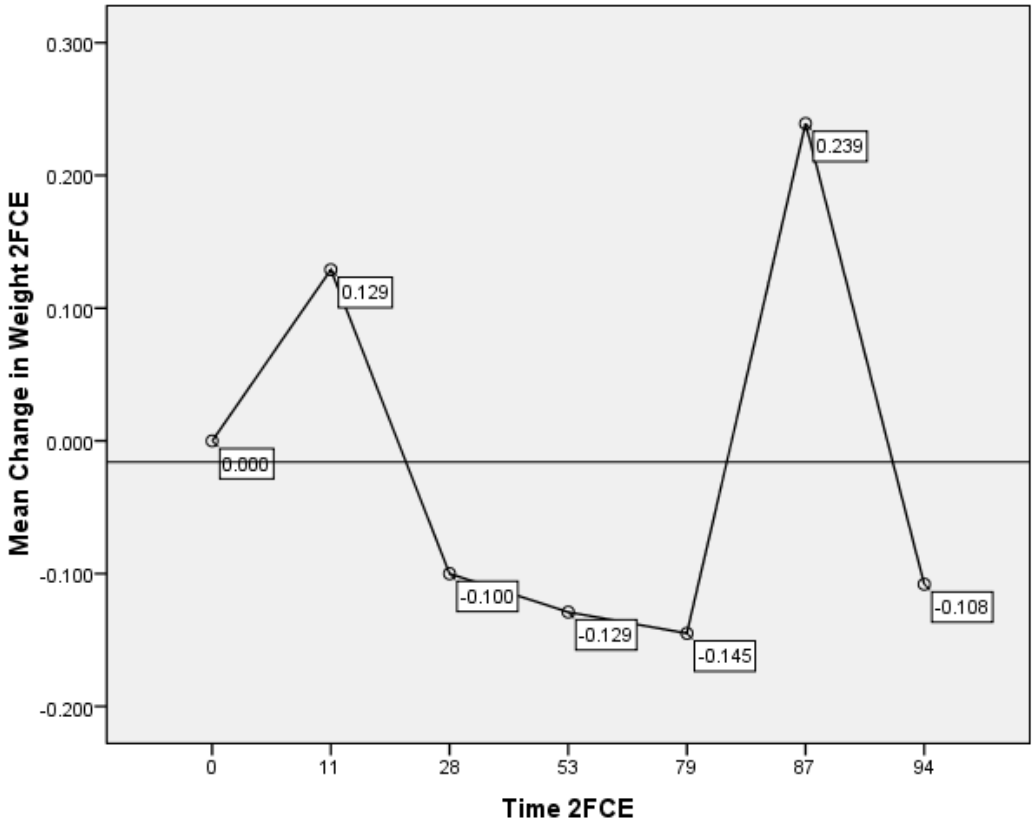


Figure 3.3 The graph shows the average weight change (grams) for the specimens in 2FCE over the duration of the experiment (94 days). The horizontal line in the graph indicates the mean weight change (-0.04 grams).

1FCE

The points for food removal and reintroduction in 1FCE were day 11 (1WWF), day 13 (1WOF), and day 26 (1WNF). The Mann-Whitney U test and the Pearson's correlation test compared the means between two days at a time. The WWF and WOF comparison had a U-value of 75.0 with a p-value of 0.126. The Pearson's correlation value was -0.05 with a significance of 0.058. This indicates the difference in the means is not statistically significant, however, these variables have a statistically significant negative correlation.

The means of 1WWF and 1WNF measurements have a U-value of 93.5 with a p-value of 0.436 and an r-value of -.0.036 with a p-value of 0.898. This indicates the difference in means and the correlation between the variables are not statistically significant.

The means of 1WOF and 1WNF have a U-value of 94.0 with a p-value of 0.461. This indicates the mean difference is not statistically significant. However, the Pearson's correlation value (r) was -0.044 with a significance of 0.10. Thus, the two variables (1WOF and 1WNF) have a negative correlation which is statistically significant.

2FCE

During 2FCE, the time points were day 79 (2WWF), day 87 (2WOF), and day 94 (2WNF). The means of 2WWF and 2WOF have a U- value of 16.0 with a p-value of 0.01. The Pearson's correlation value was -0.503 with a significance of 0.096. The mean difference is statistically significant and the negative correlation between the variables is statistically significant.

The 2WWF and 2WNF measurements have a U-value of 58.0 with a p-value of 0.414. This indicates the mean difference is not statistically significant. The Pearson's correlation r-value was 0.53 with a significance of 0.076. The correlation between these variables is a statistically significant positive relationship.

The 2WOF and 2WNF variables have a U-value of 30.0 with a p-value of 0.014. This indicates the mean difference between the day 87 and day 94 measurements is statistically significant. The Pearson's correlation value was -0.138 with a significance of 0.668. The correlation between these variables is not statistically significant.

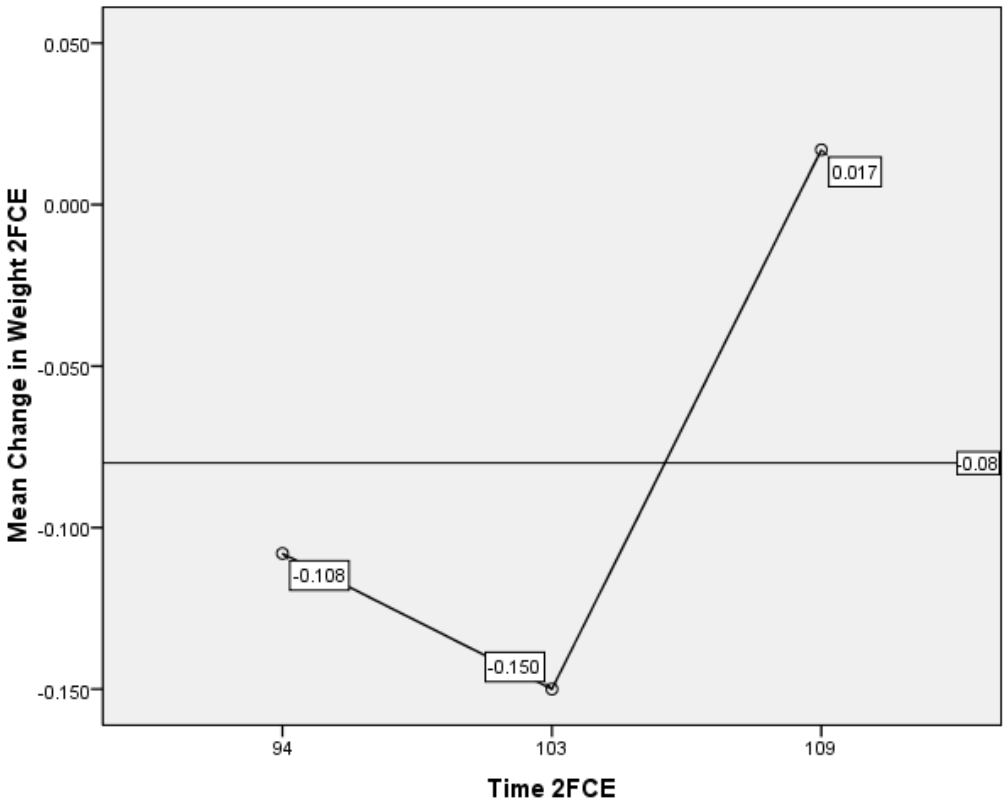


Figure 3.4 The graph shows the average weight change (grams) for the specimens in 2FCE M. ann over the duration of the experiment (15 days). The horizontal line in the graph indicates the mean weight change (-0.08 grams).

Comparing the body weight change between the feeding regimes Millepora complanata and Montastraea annularis

During a period in 2FCE (Figure 3.4 and Box 2 in Figure 3.2), *M. complanata* colonies were removed and immediately replaced with *M. annularis* colonies. The difference in body weight during three points (day 94: before introduction of *M. annularis* (2MA); day 103: after introduction of *M. annularis* (2AMA); and day 109: 15

days after introduction of *M. annularis* (2BMA)) were compared for variance and difference between the means.

Comparison of the 2MA and 2AMA means gives a U-value of 66.5 with a p-value of 0.755. This indicates the difference between the mean weights between the beginning and end of the experiment is not statistically significant. The Pearson's correlation value was -0.661 with a significance of 0.019. Therefore, the two variables have a statistically significant negative relationship.

The 2MA and 2BMA variable have a U-value of 51.0 with a p-value of 0.242. This mean difference is not statistically significant. The Pearson's correlation value was -0.032 with a significance of 0.921. The correlation between the variables is also not statistically significant.

The 2AMA and 2BMA variables have a U-value of 61.0 with a p-value of 0.551. The Pearson's correlation r-value was -0.225 with a significance of 0.481. These two measurements did not have statistically significantly different means or were correlated.

Summary of Results:

The statistically significant tests are listed in Tables 3.1 and 3.2. The difference in means (U-value) and the correlation (r-value) were both statistically significant in one series: 2FCE, weight before removal of food and weight after removal of food; (-0.503, moderately negative correlation). The U-value was statistically significant in one series where the r-value was not statistically significant:

1. 2FCE, weight after removal of *M. complanata* and weight after reintroduction of *M. complanata*.

The Pearson's correlation was statistically significant when the difference in the means was not statistically significant in four series:

1. 1FCE, weight before removal of *M. complanata* and weight after removal of *M. complanata*; (-0.5, moderately positive correlation)
2. 1FCE, weight after removal of *M. complanata* and weight after reintroduction of *M. complanata*; (-0.004, weak negative correlation)
3. 2FCE, weight before removal of *M. complanata* and weight after reintroduction of *M. complanata*; (0.53, moderately positive correlation)
4. 2FCE M.ann, weight before switch to *M. annularis* and weight 9 days after switch; (-0.661, moderately negative correlation)

Discussion:

Periodic phenomena or long-term shifts in ocean conditions can affect coral reef community ecology (May 1977, Knowlton 1992, Pandolfi et al. 2003) including the abundance and biodiversity of coral species (Bythell et al. 1993, Hughes 1994, Aronson and Precht 2001). The Caribbean is a region which has experienced local extinction and phase shifts in coral species (Hughes 1994, Macintyre et al. 2007). Important reef-building coral species like *M. annularis* have been replaced by dominate and fast-growing species like *P. astreoides* (Green et al. 2008). Moreover, critical habitat species like *A. palmata* and A. *cervicornis* are now in such low densities they are listed as

"threatened species" under the Endangered Species Act (Aronson et al. 2004). Similarly, the reef systems off of Barbados have seen a reduction in *M. annularis* and *A. cervicornis* (Macintyre et al. 2007). In contrast to the decrease in scleractinian corals, the hydrocoral *M. complanata* remains in high densities in the shallow reefs (Lewis 2006).

Table 3.1 The chart shows the Pearson's correlation tests which were statistically significant: 1FCE (days 11,13 and days 13,26); 2FCE (days 79,87 and days 79,94); 2FCE M. ann (94,103).

Pearson's Correlations: Statistically significant			
Experiment	**Days**	**r-value**	**p-value**
1FCE	11,13	-0.5	0.058
	13,26	-0.044	0.099
2FCE	79,87	-0.503	0.096
	79,94	0.053	0.076
2FCE M. ann	94, 103	-0.661	0.019

Table 3.2 The chart shows the Mann-Whitney U tests which were statistically significant: 2FCE (days 79,87 and days 87,94).

Mann-Whitney U tests: Statistically significant			
Experiment	**Days**	**U-value**	**p-value**
2FCE	79,87	16	0.001
	87,94	30	0.014

Phase shifts and mass mortality of corals can impact sessile and benthic marine organisms including the consumers of coral tissue, corallivores (Rotjan and Lewis 2008). The reduction in nutritional value and availability of corals as a food resource could have a detrimental effect on species (Baums et al. 2003b). If the new dominant coral species are less nutritious per tissue area, this may increase the area of coral tissue loss per feeding event (Baums et al. 2003b). Also, feeding behavior of corallivores (e.g. scrapers versus grazers) may not be conducive to the newly dominant coral species morphology (Rotjan and Lewis 2008). If factors are not favorable for consumption of the new coral species or if coral tissue becomes unavailable (i.e., complete coral mortality), facultative corallivores may utilize alternate food resources in larger quantities, which could have compounding impacts on the reef community (Rotjan and Lewis 2008).

Given the availability of *M. complanata* and the decreased density of other coral species as food resources for *H. carunculata* on Barbados reefs, this study evaluated the impact of consuming *M. complanata* on the body weight of the invertebrate corallivore *H. carunculata*. Two *ex situ* experiments (1FCE and 2FCE) were conducted to measure the body weight change of *H. carunculata* in an isolated feeding regime of .*M. complanata*. The average body weight at the conclusion of both experiments (1FCE and 2FCE) with *M. complanata* was less than the initial average weight. However, the difference in the means for both experiments was not statistically significant. These results indicate the body weight did not fluctuate significantly in the presence of *M. complanata.*

When the feeding regime of the second group (2FCE) was changed to the stony coral, *M. annularis*,(2FCE M. ann) the average weight decreased in 9 days from 1.36 g to 1.21 g (mean weight change = -0.15 g) followed by an increase after 15 days (1.26 g, mean weight change = 0.017 g). However, the difference in the weight means between the presence of *M. complanata and M. annularis* as the food resources was not statistically significant. Since the body weight of *H. carunculata* was not greater in the presence of *M. complanata* compared with the body weight in *M. annularis,* the null hypothesis is accepted.

Although the U-tests for all three comparisons were not statistically significant, there was a significant negative correlation between the weight in the presence of *M. complanata* and the weight 9 days after the feeding regime was switched to *M. annularis*. The results indicate the specimens' weight decreased in the presence of the new food (*M. annularis)*. However, this is one data point and afterwards the weight increased. An explanation for the initial negative correlation in weight could be a result of conditioning to one type of food (*M. annularis*). The specimens in 2FCE and 2FCE M. ann were the same and 2FCE ran for 94 days before the food was changed suddenly. In 2FCE M. ann, the specimens increased in weight after 6 more days in the *M. annularis* feeding regime which supports the idea that the worms were conditioned to feed on *M. complanata* and it took time to adjust to the new food. A control group was not used, therefore, one must be cautious in drawing conclusions from these data.

This study assumed the frequency of *H. carunculata* predation on *M. complanata* in field observations (Ott and Lewis 1972, Witman 1988, Lewis and Crooks 1996)

indicated there was a benefit to this feeding behavior. When the body weight was compared before and after starvation (1FCE and 2FCE separately), there was a statistically significant difference in the means and a negative correlation between average weights in the presence and absence of *M. complanata.* Since body weight decreased in the absence of *M. complanata* more than when colonies were available*, M. complanata* colonies provided a positive level of sustenance. However, after 26 days (1FCE) and 94 days (2FCE) of feeding, the specimens did not gain significant weight over time which indicates feeding on *M. complanata* does not provide a sustainable level of nutrition for life longevity. Regardless of the results, it is difficult to draw conclusions when comparing body weight in 1FCE and 2FCE since a control group was not used.

It is important for coral managers to know the impact of a declining resource (i.e., coral tissue) on consumers (i.e., corallivores). The experiments in this study attempted to examine the body weight of a corallivore, *H. carunculata,* in the presence of an abundant fire coral species, *M. complanata* from a system of declining scleractinian coral cover. The lack of control groups hinders the ability to draw conclusions about the results.

The experiments should have included negative and positive controls. The negative control would have tested the effect of no food. A positive control would have involved a mixed diet and a different coral species like *Porites*. There was a period of negative control for both 1FCE and 2FCE when the food was removed. However, a proper control would have occurred at the same time as the experiment with *M. complanata.* Also, in order to compare body weight under different feeding regimes (e.g. *M. complanata* versus *M. annularis*), length of experiments and number of replications of

the different food conditions should be similar. Furthermore, wet weight may not be the most comprehensive measurement to use when assessing the impact of a feeding regime on fireworm specimens. Other measurements like dry weight, length, and volume may a better set of information to determine organism growth over time.

Nevertheless, the outcome of the feeding experiments suggests *H. carunculata* does not gain weight in the presence of *M. complanata* as the only food resource despite the frequency of predation from field observations (Lewis and Crooks 1996). The same results of decreased average weight occurred during the *M. annularis* feeding regime even though the length of time under the *M. annularis* feeding regime was shorter. These results could have been from various factors including tank affects, water quality affects, seasonal growth rates, species population parameters, or nutritional value of *M. complanata.*

Life cycle affects, specifically reproduction and age, could be contributing factors to little or no weight gain. *H. carunculata* spawns in the summer months (Marsden 1962) and the experiments took place during the winter (1FCE) and early spring (2FCE). Therefore, weight gain may have been stagnant prior to the spawning season. Also, the age of the specimens may have contributed to the weight trend observed in the experiments. However, baseline information on the life cycle and growth rate as it related to life cycle of this species in not known or documented (Ritchie personal communication).

Also, body length parameters for this species are also poorly understood. Unpublished data by this author suggested the population sampled for this experiment

were smaller in average length than historic data. The specimens were taken from the population on the Batts Rock Reef off the west coast of Barbados. In 2002, this author found the average length of this population to be 4.52 cm which is a reduction from 8cm, the average size of fireworms found off the west coast of Barbados in 1962 observed by Marsden (personal communication). Comparing historic data to this unpublished data suggested this population has reduced in average growth size. The shift in average body size suggests the use of length as an indication of age would be an inaccurate measurement for this population. Therefore, more information on the life cycle of *H. carunculata* and tools to measure age are needed to determine if nutritional requirements change with age.

In a *M. complanata* feeding regime, *H. carunculata* did not increase in weight but the results from starvation periods suggest the specimens were consuming *M. complanata* and that "fire corals" does provide a certain level of nutrition. However, fire corals did not provide sufficient nutrients for growth. These data suggest *H. carunculata* needs other sources for sustenance and *M. complanata* may not be the ideal food source. Previous studies discussed the diverse diet composition of *H. carunculata* (Lewis and Crooks 1996) and Rotjan and Lewis (2008) defined this species as a facultative corallivore. If *M. complanata* is more abundant than other coral species but does not provide enough nutrients, this may affect the amount of tissue consumed per feeding event and also affect the consumption rate of alternative food sources in order for *H. carunculata* organisms to maintain nutritional requirements.

Given the declining state of reefs in the Caribbean and worldwide, more research needs to be done on the consequences for corallivores as well as their prey. Facultative corallivores, like *H. carunculata,* are less affected by a reduction in coral tissue than obligate corallivores (Rotjan and Lewis 2008). However, the consequences for alternative prey like sponges and anemones may be affected as preferred coral species, like scleractinian corals, become less abundant. Coral reef management plans should address how decline and diversity changes in scleractinian or fire corals will affect the physiology and feeding behavior of corallivores to understand the impacts on overall coral reef food webs. The first step is collecting the necessary and fundamental information on coral predators including food preference, foraging time, and diet composition.

Literature Cited

Alvarez-Filip L, Gil I (2006) Effects of Hurricanes Emily and Wilma on coral reefs in Cozumel, Mexico. Coral Reefs 25:583-583

Aronson RB, MacIntyre IG, Wapnick CM, O'Neill MW (2004) Phase shifts, alternative states, and the unprecedented convergence of two reef systems. Ecology 85:1876-1891

Aronson RB, Precht WF (2001) White-band disease and the changing face of Caribbean coral reefs. Hydrobiologia 460:25-38

Bak RPM, Steward-Van Es Y (1980) Regeneration of superficial damage in the scleractinian corals *Agaricia agaricites, f. purpurea* and *Porites astreoides*. Bull Mar Sci 30:883-887

Baums IB, Miller MW, Szmant AM (2003a) Ecology of a corallivorous gastropod, *Coralliophila abbreviata*, on two scleractinian hosts. 1: Population structure of snails and corals. Mar Biol 142:1083-1091

Baums IB, Miller MW, Szmant AM (2003b) Ecology of a corallivorous gastropod, *Coralliophila abbreviata*, on two scleractinian hosts. II. Feeding, respiration and growth. Mar Biol 142:1093-1101

Bellwood DR, Choat JH (1990) A functional analysis of grazing in parrotfishes (Family Scaridae) – the ecological implications. Environ Biol Fishes 28:189-214

Bellwood DR, Hoey AS, Choat JH (2003) Limited functional redundancy in high diversity systems: resilience and ecosystem function on coral reefs. Ecol Lett 6:281-285

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

Birkeland C, Lucas J (1990) *Acanthaster planci*: major management problem of coral reefs, Vol. CRC Press

Bythell JC, Gladfelter EH, Bythell M (1993) Chronic and catastrophic natural mortality of 3 common Caribbean reef corals. Coral Reefs 12:143-152

Chapin FS, Zavaleta ES, Eviner VT, Naylor RL, Vitousek PM, Reynolds HL, Hooper DU, Lavorel S, Sala OE, Hobbie SE, Mack MC, Diaz S (2000) Consequences of changing biodiversity. Nature 405:234-242

Chornesky EA, Peters EC (1987) Sexual reproduction and colony growth in the scleractinian coral *Porities astreoides*. Biol Bull 172:161-177

Colgan MW (1987) Coral reef recovery on Guam (Micronesia) after catastrophic predation by *Acanthaster planci*. Ecology 68:1592-1605

Connell JH (1997) Disturbance and recovery of coral assemblages. Coral Reefs 16:S101-S113

Dalton SJ, Godwin S (2006) Progressive coral tissue mortality following predation by a corallivorous nudibranch (*Phestilla* sp.). Coral Reefs 25:529-529

De Weerdt Wd (1981) Transplantation experiments with Caribbean *Millepora* species (Hyrdozoa, Coelenterata), including some ecological observations on growth forms. Bijdr Dierk 51:1-19

Edmunds PJ (1999) The role of colony morphology and substratum inclination in the success of *Millepora alcicornis* on shallow coral reefs. Coral Reefs 18:133-140

Edmunds PJ (2004) Juvenile coral population dynamics track rising seawater temperature on a Caribbean reef. Mar Ecol-Prog Ser 269:111-119

Edmunds PJ, Elahi R (2007) The demographics of a 15-year decline in cover of the Caribbean reef coral *Montastraea annularis*. Ecol Monogr 77:3-18

Edmunds PJ, Roberts DA, Singer R (1990) Reefs of the northeastern Caribbean. 1. Scleractinian populations. Bull Mar Sci 46:780-789

Folke C, Carpenter S, Walker B, Scheffer M, Elmqvist T, Gunderson L, Holling CS (2004) Regime shifts, resilience, and biodiversity in ecosystem management. Annu Rev Ecol Evol Syst 35:557-581

Gardner TA, Cote IM, Gill JA, Grant A, Watkinson AR (2003) Long-term region-wide declines in Caribbean corals. Science 301:958-960

Glynn P (1990) Feeding ecology of selected coral-reef macroconsumers: patterns and effects on coral community structure. In: Z Dubinsky (ed) Ecosystems of the world 25: coral reefs. Elsevier, New York, p 365-400

Glynn PW (1963) *Hermodice carunculata* and *Mithraculus sculptus,* two hermatypic coral predators. Association of Island Marine Laboratories 4th Meeting:16-17

Glynn PW (2004) High complexity food webs in low-diversity eastern Pacific reef-coral communities. Ecosystems 7:358-367

Green DH, Edmunds PJ, Carpenter RC (2008) Increasing relative abundance of *Porites astreoides* on Caribbean reefs mediated by an overall decline in coral cover. Mar Ecol-Prog Ser 359:1-10

Hayes JA (1990) Prey preference in a Caribbean corallivore, *Coralliophila abbreviata* (Lamark) (Gastropoda, Coralliophilidae). Bull Mar Sci 47:557-560

Hiatt R, Strasburg D (1960) Ecological relationships of the fish fauna on coral reefs of the Marshall Islands. Ecol Monogr 30:65-127

Houk P, Bograd S, van Woesik R (2007) The transition zone chlorophyll front can trigger *Acanthaster planci* outbreaks in the Pacific Ocean: Historical confirmation. J Oceanogr 63:149-154

Hughes TP (1994) Catastrophes, phase shifts, and large scale degradation of a Caribbean coral reef. Science 265:1547-1551

Hughes TP, Baird AH, Bellwood DR, Card M, Connolly SR, Folke C, Grosberg R, Hoegh-Guldberg O, Jackson JBC, Kleypas J, Lough JM, Marshall P, Nystrom M, Palumbi SR, Pandolfi JM, Rosen B, Roughgarden J (2003) Climate change, human impacts, and the resilience of coral reefs. Science 301:929-933

Hughes TP, Tanner JE (2000) Recruitment failure, life histories, and long-term decline of Caribbean corals. Ecology 81:2250-2263

Jackson JBC, Kirby MX, Berger WH, Bjorndal KA, Botsford LW, Bourque BJ, Bradbury RH, Cooke R, Erlandson J, Estes JA, Hughes TP, Kidwell S, Lange CB, Lenihan HS, Pandolfi JM, Peterson CH, Steneck RS, Tegner MJ, Warner RR (2001) Historical overfishing and the recent collapse of coastal ecosystems. Science 293:629-638

Knowlton N (1992) Thresholds and multiple stable states in coral reef community dynamics. American Zoologist 32:674-682

Knowlton N (2001) The future of coral reefs. Natl Acad Sciences, p 5419-5425

Lester RT, Bak RPM (1985) Effects of environment on regeneration rate of tissue lesions in the reef coral *Montastrea annularis* (Scleractinia). Mar Ecol-Prog Ser 24:183-185

Lewis JB (1989) The ecology of *Millepora* - A review. Coral Reefs 8:99-107

Lewis JB (1992) Heterotrophy in corals – zooplankton predation by the hydrocoral *Millepora complanata.* Mar Ecol-Prog Ser 90:251-256

Lewis JB (2006) Biology and ecology of the hydrocoral *Millepora* on coral reefs. In: Advances in Marine Biology, Vol 50, Vol 50. Academic Press Ltd, London, p 1-55

Lewis JB, Crooks RE (1996) Foraging cycles of the amphinomid polychaete *Hermodice carunculata* preying on the calcareous hydrozoan *Millepora complanata*. Bull Mar Sci 58:853-856

Lizama J, Blanquet RS (1975) Predation on sea anemones by the amphinomid polychaete, *Hermodice carunculata*. Bull Mar Sci 25:442-443

Lokrantz J, Nystrom M, Thyresson M, Johansson C (2008) The non-linear relationship between body size and function in parrotfishes. Coral Reefs 27:967-974

Macintyre IG, Glynn PW, Toscano MA (2007) The demise of a major *Acropora palmata* bank-barrier reef off the southeast coast of Barbados, West Indies. Coral Reefs 26:765-773

Marsden JR (1963) The digestive tract of *Hermodice carunculata.* (Pallas). Polychaeta: Amphinomidae. Can J Zool/Rev Can Zool 41:165-184

Marsden JR (2001) personal communication, unpublished data.

Martin A, Losada F (1991) Regeneration of injuries caused by the Amphinomid polychaete *Hermodice carunculata* on scleractinian corals, hydrocorals and octocorals (Cnidaria). Ecotropicos 4:18-26

May RM (1977) Thresholds and multiple stable states in coral reef community dynamics. Nature 269:471-477

McGuire MP (1998) Timing of larval release by *Porites astreoides* in the northern Florida Keys. Coral Reefs 17:369-375

Miller MW, Williams DE (2007) Coral disease outbreak at Navassa, a remote Caribbean island. Coral Reefs 26:97-101

Moran PJ (1986) The *Acanthaster* phenomenon. Oceanogr Mar Biol 24:379-480

Morton B, Blackmore G, Kwok CT (2002) Corallivory and prey choice by *Drupella rugosa* (Gastropoda : Muricidae) in Hong Kong. J Molluscan Stud 68:217-223

Motta PJ (1988) Functional morphology of the feeding apparatus of 10 species of pacific butterflyfishes (Perciformes, Chaetodontidae) – an ecomorphological approach. Environ Biol Fishes 22:39-67

Mumby PJ, Hastings A, Edwards HJ (2007) Thresholds and the resilience of Caribbean coral reefs. Nature 450:98-101

Myers RA, Worm B (2003) Rapid worldwide depletion of predatory fish communities. Nature 423:280-283

Oren U, Benayahu Y, Loya Y (1997) Effect of lesion size and shape on regeneration of the Red Sea coral *Favia favus*. Mar Ecol-Prog Ser 146:101-107

Ott B, Lewis JB (1972) The importance of the gastropod *Coralliophila abbreviata* (Lamarck) and the polychaete *Hermodice carunculata* (Pallas) as coral reef predators. Can J Zool/Rev Can Zool 50:1651-1656

Pandolfi JM, Bradbury RH, Sala E, Hughes TP, Bjorndal KA, Cooke RG, McArdle D, McClenachan L, Newman MJH, Paredes G, Warner RR, Jackson JBC (2003) Global trajectories of the long-term decline of coral reef ecosystems. Science 301:955-958

Richardson LL, Voss JD (2005) Changes in a coral population on reefs of the northern Florida Keys following a coral disease epizootic. Mar Ecol-Prog Ser 297:147-156

Ritchie K (2009) personal communication, unpublished data.

Rotjan RD, Dimond JL, Thornhill DJ, Leichter JJ, Helmuth B, Kemp DW, Lewis SM (2006) Chronic parrotfish grazing impedes coral recovery after bleaching. Coral Reefs 25:361-368

Rotjan RD, Lewis SM (2008) Impact of coral predators on tropical reefs. Mar Ecol-Prog Ser 367:73-91

Runnalls LA, Coleman ML (2003) Record of natural and anthropogenic changes in reef environments (Barbados West Indies) using laser ablation ICP-MS and sclerochronology on coral cores. Coral Reefs 22:416-426

Sheppard CRC (1982) Coral populations on reef slopes and their major controls. Mar Ecol-Prog Ser 7:83-115

Sussman M, Loya Y, Fine M, Rosenberg E (2003) The marine fireworm *Hermodice carunculat*a is a winter reservoir and spring-summer vector for the coral-

bleaching pathogen *Vibrio shiloi*. Environmental microbiology, 2003 Apr, 5(4):250-5

Sutherland KP, Porter JW, Torres C (2004) Disease and immunity in Caribbean and Indo-Pacific zooxanthellate corals. Mar Ecol-Prog Ser 266:273-302

Tomascik T, Sander F (1987) Effects of eutrophication on reef-building corals. 2. structure of scleractinian coral communities on fringing reefs, Barbados, West Indies. Mar Biol 94:53-75

Turner SJ (1994) The biology and population outbreaks of the corallivorous gastropod *Drupella* on Indo-Pacific reefs. In: Oceanography and Marine Biology, Vol 32, Vol 32. U C L Press Ltd, London, p 461-530

Wahle CM (1980) Detection, pursuit, and overgrowth of tropical gorgonians by *Milleporid* hydrocorals - Perseus and Medusa revisited. Science 209:689-691

Williams DE, Miller MW (2005) Coral disease outbreak: pattern, prevalence and transmission in *Acropora cervicornis*. Mar Ecol-Prog Ser 301:119-128

Witman JD (1988) Effects of predation by the fireworm *Hermodice carunculata* on *Milleporid* hydrocorals. Bull Mar Sci 42:446-458

Witman JD (1992) Physical disturbance and community structure of exposed and protected reefs - a case study from St. John, US Virgin Islands. American Zoologist 32:641-654

4. The use of molecular techniques to investigate the feeding behavior of *Hermodice carunculata*

Abstract

The invertebrate corallivore *Hermodice carunculata* was associated with the tissue loss margin of coral colonies during the 2005, 2006, and 2007 winter outbreaks of a suspect white plague (WP) in the Flower Garden Banks National Marine Sanctuary. However, the gross signs of denuded tissue were inconsistent with WP-associated lesions. During the winter outbreak in 2007, actively feeding *H. carunculata* organisms were foraging on tissue loss margins. The tissue loss margins of colonies without actively feeding exhibited patterns of tissue loss inconsistent with WP. Lesions caused by *H. carunculata* also result in white denuded skeleton. However, specific information on predation patterns and scars is unavailable for *H. carunculata.* Diagnosis techniques and protocols are needed to distinguish *H. carunculata*-induced tissue loss from microbial-induced tissue loss. This study tested the ability to detect trace amounts of *H. carunculata* DNA on known predation sites by developing specific *H. carunculata* primers to amplify its DNA using Polymerase Chain Reactions. Under the conditions of this study, the *H.carunculata* DNA was not detectable. However, appropriately adjusted techniques should be useful to develop comprehensive and accurate field diagnostic tools of coral tissue mortality.

Introduction

Coral diseases caused by infectious microorganisms are considered the most important factor contributing to the decline of live coral tissue and coral population densities (Harvell et al. 1999, Wilkinson 2004, Bruckner and Bruckner 2006, Weil and Croquer 2009). Over the past 30 years, coral diseases have increased in range (Weil et al. 2002, Sutherland et al. 2004, Raymundo et al. 2005) and prevalence (Bruno et al. 2003, Sutherland et al. 2004), subsequently damaging reefs worldwide (Harvell et al. 1999, Porter et al. 2001, Rosenberg and Loya 2004). It has been more than 30 years since field observers first noticed unusual lesions on corals (Weil et al. 2006), but the identities of most pathogens remain unknown (Richardson 1998, Harvell et al. 2007, Luna et al. 2007, Weil and Croquer 2009).

Of the 29 proposed coral diseases (Remily and Richardson 2006) that lead to partial or complete colony mortality, only five microbial causal agents have been implicated through Koch's postulates (Sutherland et al. 2004, Harvell et al. 2007): white plague type II, white pox, aspergillosis, *Vibrio shiloi*-induced bleaching, and *Vibrio coralliilyticus*-induced bleaching and disease (Table 4.1). The causative agent of white plague II (*Aurantimonas coralicida*) was not been confirmed since Richardson et al (1998c). Several coral diseases (black band disease (BB), shut-down reaction (SD), skeletal anomalies (SA), aspergillosis (AS), white pox (WP), dark spots (DS), *Vibrio shiloi*-induced bleaching (VS), *Vibrio coralliilyticus*-induced bleaching and disease (VC), yellow band (YB), and fungal-protozoan syndrome (FP)) have also been correlated with various abiotic stressors including elevated sea temperature, eutrophication,

sedimentation, pollution, fecal contamination, solar UV radiation, and poor water quality (Table 4.2).

Table 4.1 Coral diseases associated with biota which have been proven through Koch's postulates listed by disease, biota, and literature reference.

Disease	**Biota**	**Reference**
White Plague Type II	*Aurantimonas coralicida*	Richardson et al. 1998a, Denner et al. 2003
White Pox	*Serratia marcescens*	Patterson et al. 2002
Aspergillosis	*Aspergillus sydowii*	Smith and Weil 2004, Geiser et al. 1998
Vibrio shiloi-induced Bleaching	*Vibrio shiloi*	Kushmaro et al., 1997, Rosenberg et al. 1998
Vibrio coralliilyticus-induced Bleaching	*Vibrio coralliilyticus*	Ben-Haim and Rosenberg 2002, Ben-Haim et al. 2003

Due to the plethora of phenomena contributing to coral reef decline, it is difficult to determine the importance of each factor in the survivorship, abundance, and biodiversity of coral species (Bythell et al. 1993, Hughes 1994, Aronson and Precht 2001, Knowlton 2001, Gardner et al. 2003, Edmunds and Elahi 2007). The ability of coral colonies to recover from detrimental events, like epizootics or long-term shifts in ocean conditions, is based on various intrinsic factors including species body size (Bak and Meesters 1998, Kramarsky-Winter and Loya 2000), morphology (Nagelkerken and Bak 1998), and age (Henry and Hart 2005). Extrinsic factors which also limit regeneration capabilities include wound characteristics (Henry and Hart 2005), water temperatures (Kramarsky-Winter and Loya 2000), food availability (Nagelkerken et al. 1999), and disturbance history (Cumming 2002).

Table 4.2 Coral diseases associated with abiotic stressors listed by disease, stressor, and literature reference.

Disease	**Abiotic Stressor**	**Reference**
Black Band Disease	Elevated Temperatures Eutrophication Sedimentation Pollution Fecal contamination	Richardson and Kuta 2003 Kuta and Richardson 2002 Frias-Lopez et al. 2002 Al-Moghrabi 2002 Frias-Lopez et al. 2002
Shut-Down Reaction	Elevated Temperatures Sedimentation	Antonius 1977 Antonius 1977
Skeletal Anomalies	Solar UV Radiation	Coles and Seapy 1998
Aspergillosis	Elevated Temperatures Sedimentation Poor water quality	Alker et al. 2001 Shinn et al. 2000, Smith and Weil 2004 Kim and Harvell 2002
White Pox	Elevated Temperatures Fecal contamination	Patterson et al. 2002 Patterson et al. 2002
Dark Spots	Elevated Temperatures	Gil-Agudelo and Garzon-Ferreira 2001
Vibrio shiloi-induced bleaching	Elevated Temperatures	Israely et al. 2001
Vibrio coralliilyticus-induced bleaching and disease	Elevated Temperatures	Ben-Haim et al. 2003
Yellow Band	Elevated Temperatures	Riegl 2002
Fungal-Protozoan Syndrome	Elevated Temperatures	Cerrano et al. 2000

To understand the role of coral disease in the state of coral reef ecosystems, other threats to tissue loss, in particular predation, need to be better understood and distinguished from disease-induced tissue loss (Miller and Williams 2007). However, using field observations to determine causal agents of coral tissue loss is understood to be

a major challenge (Zorpette 1995, Bruckner 2006, Ainsworth et al. 2007). Coral tissue consumed by corallivores such as *Hermodice carunculata* (bearded fireworm), *Coralliophila abbreviata* spp., *Drupella* spp. (corallivorous gastropod), and *Acanthaster planci* (crown-of-thorns starfish) leave denuded skeleton lesions similar in appearance to those attributed to pathogenic microorganisms (Witman 1988, Sussman et al. 2003, Miller and Williams 2007) and the lesions can easily be mistaken as microbial disease (Sutherland et al. 2004). Thus, effective and accurate diagnostic tools are needed to distinguish disease-induced tissue loss from corallivory (Miller and Williams 2007).

Identifying predation patterns is also important to understand the impact of coral predators on an already declining system (Bruckner 2001, Rosenberg and Loya 2004, Rotjan and Lewis 2008). The diet composition of corallivores has a 100% coral species overlap (including many scleractinians), which could exacerbate the decline of the preferred coral species (Rotjan and Lewis 2008). Chronic corallivory may cause the affected colonies to redirect energy for repair which would otherwise be used for reproduction and growth (Henry and Hart 2005). Furthermore, predation can also increase susceptibility to colonization of algal turfs which can inhibit coral recovery and increase incidences of disease (Rotjan and Lewis 2005). Field studies have reported progressive coral tissue loss after corallivores leave foraging sites which suggests the predator might transmit pathogenic bacteria to healthy corals (Sussman et al. 2003, Williams and Miller 2005, Dalton and Godwin 2006). The role of corallivores in coral tissue loss needs further study because of this distinct possibility that corallivores are

disease vectors, as well as being predators threatening coral survival (Rosenberg and Loya 2004, Sutherland et.al. 2004, Williams and Miller 2005).

This study focused on one of the 116 known corallivorous species (Rotjan and Lewis 2008), the amphinomid polychaete *H. carunculata* (Marsden 1963). As a facultative corallivore (Rotjan and Lewis 2008), this species eats scleractinian corals and also grazes on zoanthids, anemones, gorgonians, and hydrocorals (Glynn 1963, Marsden 1963, Ott and Lewis 1972, Lizama and Blanquet 1975, Martin and Losada 1991). Field observations of Miller and Williams (2007) and others (S. Lewis, G. Cook, R. Jonas, unpub. observ.) contradict literature reports that fireworms feed exclusively during late afternoon and early evening (Marsden 1962, Lewis and Crooks 1996, Witman 1988). Wittman (1988) found *H. carunculata* population densities differed regionally, but were consistent within each region. This species locates its prey through a chemoreceptor located immediately dorsal to its mouth region, the nuchal organ (Martin and Losada 1991). *H. carunculata* mechanically removes coral tissue by everting its muscular pharynx over the prey (Marsden 1962, Miller and Williams 2007). Through the use of its strong muscular buccal region (Ott and Lewis 1972), the organism sucks the tissue into the esophagus and may excrete digestive enzymes to aid in removal of tissue (Marsden 1962, Marsden 1963, Ott and Lewis 1972, Witman 1988). This species has been observed to consume 12.9 cm^2 $d^{-1}$of coral tissues (Rotjan and Lewis 2008) and have two separate feeding episodes before retreating into reef cervices (Lewis and Crooks 2006). Once ingested, particles can remain in the pharynx for up to nine hours (Marsden 1968). Larger lesions are correlated with longer foraging times and larger body size (Ott and Lewis

1972). A foraging event is apparent by the noticeable enlargement of the organism's buccal region and its sedentary orientation (Marsden 1962, S. Lewis, unpublished data). It is difficult to determine the correlation of *H. carunculata* with other pathogens through field observations, because gross patterns of *H. carunculata* tissue removal from scleractinian coral species have not been documented in the literature either photographically or descriptively (Miller and Williams 2007). *Ex situ* experiments have shown *H. carunculata* to be a vector for the *Vibrio shiloi*-induced bleaching pathogen (Sussman et al. 2003).

The feeding behavior of *H. carunculata* on reefs of the Flower Garden Banks National Marine Sanctuary (FGBNMS) was investigated in relation to outbreaks of the white syndrome most often referred to as white plague (WP). Preliminary unpublished field studies on *H. carunculata* by this author indicated the lesions of rapid tissue loss in suspect WP are similar to the gross patterns of tissue removal resulting from predation by the invertebrate corallivore *H. carunculata* (Figure 4.1). All suspect WP-type lesions exhibit a smooth margin along apparently healthy, but receding, coral tissue and stark white, recently denuded skeleton (Bruckner 2001, Bythell et al. 2004) (Figure 4.2). Lesions caused by *H. carunculata* also result in white denuded skeleton (Ott and Lewis 1972, Miller and Williams 2007). At least 38 coral species are susceptible to WP or WP-like diseases (Bruckner 2006, Bythell et al. 2004, Sutherland et al. 2004). Dustan (1977) initially described this as a slow progressing, lethal disease of stony corals and referred to it as WP (now WP type I), but no etiological agent was described. Since then, two more damaging, faster progressing forms of this disease have been identified: WP types II and

III (WPII and WPIII) (Richardson et al.1998a). The diagnostic criteria for WP have been complicated by inconsistent results of identification of bacterial etiological agents, including finding different bacteria associated with the diseased margin (Denner et al. 2003, Pantos et al. 2003). The inconclusive findings of causal agents of WP (Ainsworth et al. 2007, Richardson et al. 1998a, Rosenberg and Loya 2004) and the recent predation correlations with suspect white plague outbreaks (Miller and Williams 2007) indicates more studies are needed on WP and predation at the tissue loss margin.

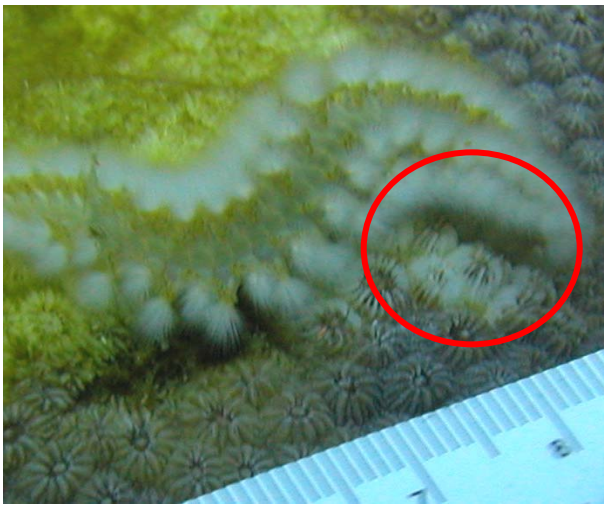


Figure 4.1. *H. carunculata* feeding on a *Montastraea* colony denuded skeleton margin in FGBNMS in winter 2007. The red circle highlights recently denuded tissue from this foraging event. Photo by R.B. Jonas.

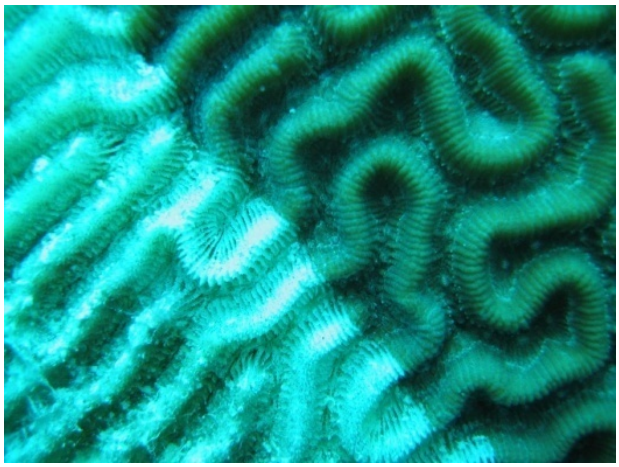


Figure 4.2. Suspect white plague killing a colony of *Diploria strigosa,* Loee Key, Florida Keys National Marine Sanctuary, July 2006. Photo by E. Peters

A suspect WP-like outbreak occurred during the winters of 2005, 2006, and 2007 on reefs of the FGBNMS. Field observations of the tissue loss margins suggested predation by the fireworm *H. carunculata* might be responsible for some of the tissue loss. Numerous *H. carunculata* were feeding during daylight hours (1000 to 1400 h) on denuded tissue margins of coral colonies, but not on apparently healthy colonies (Figure 4.3) during winter 2007. During surveys in November 2004, Miller and Williams (2007) also observed *H carunculata* feeding on scleractinian species affected with "white disease" signs and no organisms feeding on disease-free corals at several sites around the uninhabited island of Navassa. The observed feeding events in Navassa and FGBNMS were inconsistent with previous studies by Lewis and Crooks (1996) and J.B. Lewis (unpub. data) who found the typical foraging cycle for this organism during the late afternoon to early evening. In addition, previous observations of fireworm populations on

similar reefs (Witman 1988, Lewis and Crooks 1996) are inconsistent with the densities of foraging *H. carunculata* during the white syndrome outbreaks in Navassa and the FGBNMS, which suggests a dramatic increase in the populations. Population explosions (or outbreak) of other corallivores, including the crown-of-thorns starfish (*Acanthaster)* in the Indo-Pacific, have proven devastating to coral reefs (Volger et al. 2008). On closer examination in FGBNMS and Navassa, the tissue loss patterns were not completely consistent with those of WP (R.B. Jonas personal communication, Miller and Williams 2007). Furthermore, the white syndrome outbreaks occurred during the colder seawater temperatures when most microbial coral diseases are not active (Bruno et al. 2007).

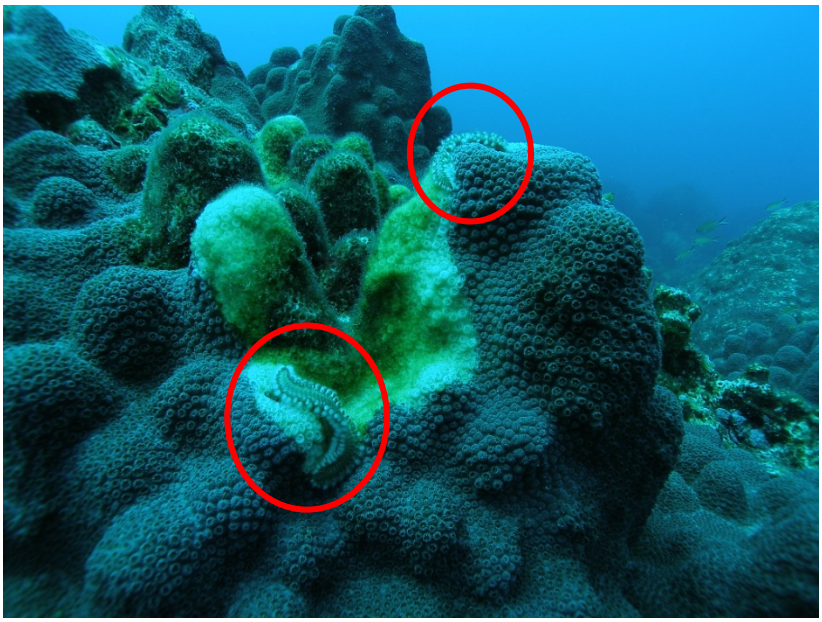


Figure 4.3. Two *H. carunculata* specimens (indicated by red circles) feeding on a *Montastraea* colony at the tissue loss margin during the white syndrome outbreak in FGBNMS in winter 2007. Photo by M/V *Spring* staff.

Diagnostic tools are needed to determine when tissue loss is resulting from *H. carunculata* predation (and by extension, by other predators or pathogens). This study assessed the feeding behavior of *H. carunculata* in relation to the white syndrome event in the FGBNMS during the winter of 2007. Using known *H. carunculata* foraging sites, samples were collected to determine the accuracy of molecular markers as diagnostic tools of *H. carunculata*-induced lesions. Given the direct contact of predator to prey, I hypothesized that *H. carunculata* leaves a trace amount of DNA at feeding sites, which can be identified through DNA amplification. The null hypothesis was *H. carunculata* does not leave a trace molecular marker at foraging sites.

Methods

Sample Collection

The collection sites were the East Bank (FE) (27°54′33.0″ latitude and 93°35′59.7″ longitude) and West Bank (FW) (27°52′30.6″ latitiude and 93°48′54.1″ longitude) of the Flower Garden Banks National Marine Sanctuary (FGBNMS) from March 4-9, 2007. On March 5, a dive took place on buoy 5 (FW511) at a depth of 71 ft and the water temperature of 68 °F. A dive was conducted on March 6 at a depth of 71ft with the water temperature was 68 °F on the West Bank at buoy 5 (FW501). On March 7, 2007, a 67 foot dive at a temperature of 67 °F was conducted on buoy 4 (FE401). On March 8, 2007, a dive at a depth of 65ft with 68°F water temperature was conducted on the East Bank at buoy 2 (FE201). As the coral species most commonly exhibiting signs of apparently rapid tissue loss, *Montastraea annularis* coral colonies with active predation

were targeted for collection. First, *H. carunculata* actively feeding on coral tissue margins were removed from the feeding site, swabbed, and collected for laboratory analysis. Then swabs and cores were taken from the tissues of the same coral colonies exhibiting tissue loss and active predation by *H. carunculata*. The samples were labeled according to the collection site (FE201, FE401, FW501, or FW511). When possible, three cores and swabs were taken along the tissue loss margin: 10cm on both sides of feeding site along the tissue loss margin and one sample from the foraging site. Three cores and swabs (control) were taken from seemingly healthy tissue on the diseased colony.

Swabbing Technique:

Sterile, synthetic-topped swabs were used to obtain cells and external or internal secretions (e.g., mucus) from both actively feeding fireworms and the foraging sites on corals prior to collecting coral core samples. To prepare swabs for sampling, the ends were trimmed to fit into a 9-mL sterilized, air-filled glass tube (one swab per tube). Once the collection site had been identified, divers put on surgical, latex gloves. The designated swab was pulled from the inverted tube for immediate sampling. All swabs were promptly returned to their mostly air-filled glass tube. Exposure of the sterile swab to seawater was minimized by injecting small volumes of compressed air into the inverted test tube.

First, the targeted fireworm was removed from its foraging site using sterilized forceps and its mouth was immediately swabbed. The fireworm was then placed into a

50-mL tube (Falcon™ tubes – BD Biosciences) with ambient seawater and transported to the surface. The swabs intended for coral sampling were removed from their tube and rubbed vigorously over the surface tissue for 4 seconds. Swab samples were collected from coral tissue at the site of active feeding, 10 cm on each side of the site along the tissue loss margin, and from the surface of an apparently healthy tissue area on the affected coral.

Coring Technique:

Coral tissue and the associated skeleton was collected using sterilized, seamless, stainless steel corers carried to the sample site in sterile, screwcapped, 50-mL tubes (Falcon™ tubes – BD Biosciences) filled with 0.2-µm filtered seawater. After sampling with swabs, the divers removed the designated corer from the sterile tube. The corer was placed sharpened end down on the surface of the coral colony. Using a sledge hammer, the diver penetrated the coral colony with the corer (see Figure 4.4). The corer was tapped on both sides to ensure a clean break from the skeleton and gently pulled out of the colony. After each sample had been acquired, the stainless steel corer containing the coral tissue and skeleton was immediately returned to its dedicated 50-mL Falcon™ transport tube and the steps were repeated for each core sample. The coral disk remained in the core tube until processing could commence on the surface. The small divots created by the coring procedure were plugged with waterproof Roma Plastalina No. 2 clay immediately after sampling.

Figure 4.4: A diver extracts 1.6-cm diameter cores of coral tissue and skeleton by hammering seamless, stainless steel tubes into select areas of a colony during 2005 collection. Photo by R.B. Jonas.

Sample handling:

Some swab samples were lost by the divers during the return to the ship after sampling. The full suite of swab samples (7 in total) was obtained for FE201. The fireworm mouth swab samples were not recovered from FE401 and FW511. Only one swab was obtained from the healthy section of corals on FW511 and 2 swabs from the healthy section were obtained from FW501. Four coral colonies exhibiting WP-like disease with active *H. carunculata* predation were sampled to obtain a total of 23 swabs: 2 from the mouth of an actively feeding *H. carunculata* (MS1), 4 from active feeding sites on the tissue loss margin (FS1P), 8 from the tissue loss margin 10 cm away from feeding sites (FS2, FS3), and 9 from seemingly healthy tissue on sampled colonies (HS1,

HS2, HS3). The summary of swab samples is provided in Table 4.3. Swab stems were trimmed to fit and then placed into dedicated cryovials, frozen at liquid nitrogen temperature (-196 °C), and returned to the laboratory in a thermal-regulated dry shipper.

The colonies used for the swab sampling were also cored for a total of 16 cores: 4 from active feeding sites on the tissue loss margin (FC1P), 4 from the tissue loss margin 10 cm away from feeding sites (FC2, FC3), and 8 from seemingly healthy tissue on sampled colonies (HC1, HC2, HC3). The summary of core samples is provided in Table 4.4. Weather conditions prevented collecting a full suite of core samples from FE201 and FE401. Only two cores were collected from these sites: one at the feeding site and one from the apparently healthy surfaces of the coral colonies. On the ship, the cores were removed from the Falcon™ tubes and trimmed with a diamond blade on a dremel tool to reduce the quantity of skeleton. The samples were placed in individual cryovials and frozen at liquid nitrogen temperature (-196 °C) for return to the laboratory using a thermal-regulated dry shipper.

The actively feeding fireworms were taken to the ship in individual Falcon™ tubes containing ambient seawater. Each one was removed from the tube and dissected immediately into three parts: anterior (head), trunk, and posterior. Each part was further cut sagittally and placed in a labeled cryovial, then frozen at liquid nitrogen temperature (-196 °C) for return to the laboratory in the thermal-regulated dry shipper.

Analysis

DNA Extraction and test for eukaryotic 18S ribosomal RNA

The genomic DNA of fireworms and coral core and swab samples was extracted using a refined protocol based on the Bio101 FastDNA Extraction kit for tissue. Prior to extraction, the *H. carunculata* samples were removed from the freezer and allowed to thaw completely in the refrigerator. Approximately 200 mg tissue was cut from each collected *H. carunculata* specimens and placed into individual lysing tubes for DNA extraction. Each frozen coral core samples was crushed using a sterilized mortar and pestle and allowed to thaw afterwards in the refrigerator. Once thawed, an approximate 50/50 mix of liquid and solid portions (total of 200 µl) of each sample was pipetted into the designated lysing buffer tube for extraction. To prepare the swab samples for DNA extraction, each sample was placed in its designated lysing tube and soaked in 1 mL of lysing buffer for 1 hour.

After extraction, an aliquot of sample DNA was amplified with a universal protist 18S primer to determine whether eukaryotic DNA was present. Fam_Prot18SF was used as the protist primer to amplify the 18S rRNA of eukaryotic organisms. The reverse primer was DA443R. DNA extracted from American Type Culture Collection protist CCMP768 (*Rhodomonas* sp.) was used as a positive control during the initial PCR runs. Each PCR reaction went through 35 cycles of the PCR using *Taq* Gold polymerase with an annealing temperature of 54 °C. PCR products were run on a 1% agarose electrophoresis gel to quantify and size the amplification product.

Table 4.3 Swab Sample Summary. The first column indicates the sample site, second column is the sample number, and the third column is the description of each sample.

SITE	SAMPLE	TYPE
FE201	MS1	swab from fireworm's mouth
	FS1P	swab from site of H1 feeding fireworm
	FS2	swab from margin 10cm away from feeding fireworm
	FS3	swab from margin 10cm away from feeding fireworm
	HS1	swab from healthy part of colony where fireworm was feeding
	HS2	swab from healthy part of colony where fireworm was feeding
	HS3	swab from healthy part of colony where fireworm was feeding
FE401	FS1P	swab from site of H1 feeding fireworm
	FS2	swab from margin 10cm away from feeding fireworm
	FS3	swab from margin 10cm away from feeding fireworm
	HS1	swab from healthy part of colony where fireworm was feeding
	HS2	swab from healthy part of colony where fireworm was feeding
	HS3	swab from healthy part of colony where fireworm was feeding
FW501	MS1	swab from fireworm's mouth
	FS1P	swab from site of H1 feeding fireworm
	FS2	swab from margin 10cm away from feeding fireworm
	FS3	swab from margin 10cm away from feeding fireworm
	HS1	swab from healthy part of colony where fireworm was feeding
	HS3	swab from healthy part of colony where fireworm was feeding
FW511	FS1P	swab from site of H1 feeding fireworm
	FS2	swab from margin 10cm away from feeding fireworm
	FS3	swab from margin 10cm away from feeding fireworm
	HS1	swab from healthy part of colony where fireworm was feeding

Table 4.4 Cores Sample Summary. The first column indicates the sample site, second column is the sample number, and the third column is the description of each sample.

SITE	SAMPLE	TYPE
FE201	FC1P	core from site of H1 feeding fireworm
	HC1	core from healthy part of colony where fireworm was feeding
FE401	FC1P	core from site of H1 feeding fireworm
	HC1	core from healthy part of colony where fireworm was feeding
FW501	FC1P	core from site of H1 feeding fireworm
	FC2	core from margin 10cm away from feeding fireworm
	FC3	core from margin 10cm away from feeding fireworm
	HC1	core from healthy part of colony where fireworm was feeding
	HC2	core from healthy part of colony where fireworm was feeding
	HC3	core from healthy part of colony where fireworm was feeding
FW511	FC1P	core from site of H1 feeding fireworm
	FC2	core from margin 10cm away from feeding fireworm
	FC3	core from margin 10cm away from feeding fireworm
	HC1	core from healthy part of colony where fireworm was feeding
	HC2	core from healthy part of colony where fireworm was feeding
	HC3	core from healthy part of colony where fireworm was feeding

Testing for H. carunculata DNA in the samples

A nucleotide sequence analysis was conducted to design primers for *H. carunculata* DNA in the FGBNMS samples. First, the nucleotide sequence for the *H. carunculata* was obtained from GenBank and compared using Sequencher (Gencodes,

Ann Arbor, MI) with the nucleotide sequence of *M. annularis* to select a sequence of bases that did not have similarities to *M. annularis* and were highly selective for *H. carunculata*. Two reverse primers were designed using Primer Select (DNAStar, Madison, WI) and paired with two forward protist primers to create a long product of 526 base pairs using Prot18SF (5' GGTTGATCCTGCCAGTAGTCATATGCTTG 3', T_m = 61 °C) and HC737RA (5' AGTTAAGAGCACCAAGGGAAAACCGG 3', T_m = 60 °C) and a short product of 260 base pairs using Prot397F (5' CCGGAGAGGGAGCCTGA 3', T_m = 54 °C) and HC737RB (5' TAAGAGCACCAAGGGAAAACCGG 3', T_m=57 °C). The four primers lacked self-complementary sequences or hairpins.

Table 4.5 Standard PCR Protocol with *Taq* Gold DNA Polymerase used for all the PCR reactions.

Reagents	**Volume (µl)**
DEPC water	7.9
10X PCR Gold Buffer	2
25mM Magnesium mix	2
dNTPs (2mM each)	2
0.1% BSA	2
Forward Primer	1
Reverse Primer	1
Taq DNA Polymerase	0.1
Master Mix Volume	18
DNA from Sample	2
Final Volume	20

All the swab and core samples, except for the three which did not have quantifiable DNA, were processed through PCR with the two sets of primers specific to *H. carunculata*. Each PCR reaction ran through 35 cycles with *Taq* Polymerase. The positive control reactions used DNA extracted from *H. carunculata* tissue. The products were removed from the PCR machines and electrophoresed on a 1% agarose gel.

Results

All 43 samples (including 4 *H. carunculata* tissue samples) were tested for the presence of eukaryotic DNA by using a universal protist primer, Fam_Prot18SF and DA443R (see Figure 3.5). Three out of 43 samples (FW501H1, FW511MS1, FE201MS1) did not amplify with the universal protist primer. Therefore these samples did not have detectable amount of DNA and were not used to test for *H. carunculata* DNA.

The remaining 40 samples from the FGBNMS sites were run through a series of PCR tests with the long (Prot18SF-Hc737RA) and the short (Prot397F-Hc737RB) primer sets designed to select for *H. carunculata* DNA. The 3 samples from *H. carunculata* tissue that had detectable amounts of DNA (FE201H1, FE401H1, FW501H1) were used as positive controls in the reactions. Each sample was run through a PCR with both *H. carunculata*-specific primers at least twice to ensure the results were consistent. In all cases, the positive controls were the only samples where the DNA was amplified in the PCR tests (see Figure 3.6).

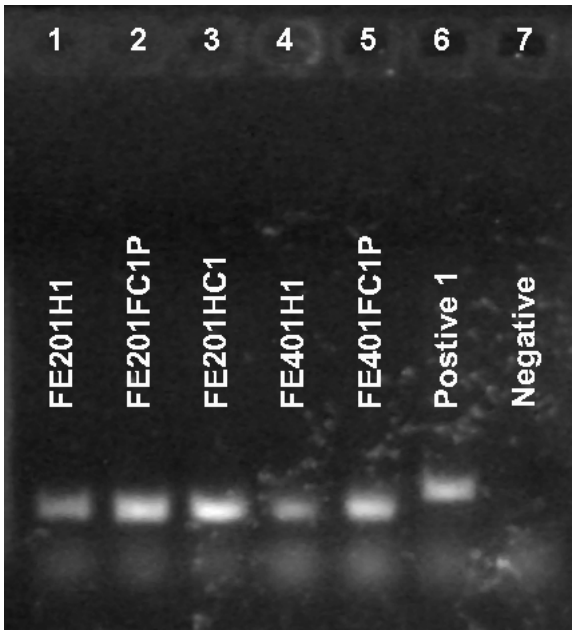


Figure 4.5 PCR with Universal Protist Primer**.** The agarose gel in this picture shows the PCR reaction using the protist primer FAM_Prot18SF (DA443R). The positive in the reaction was the protist CCMP768 and the negative was absent of DNA in the reaction tube. The DNA in the core (FE201FC1P and HC1, FE401FC1P) and tissue samples (FE201H1 and FE401H1) amplified in the presence of the primer.

Despite direct contact with the *H. carunculata* species, the DNA on the mouth swabs (FE201MS1, FE401MS1, FW501MS1, FW511MS2), was not amplified by the *H. carunculata*-specific primers. Since all the samples, except for the *H. carunculata* tissue samples, were in the direct contact with coral, the negative PCR results may be caused by an inhibition of *H. carunculata* by coral DNA. To preclude this problem, a PCR was run with a different ratio of *H. carunculata* tissue DNA and coral DNA extracted from healthy coral cores (1:1, 1:2, 1:10) (see Figure 3.7). The PCR products demonstrated that even in the highest ratio of coral DNA (1:10), *H. carunculata* DNA was amplified.

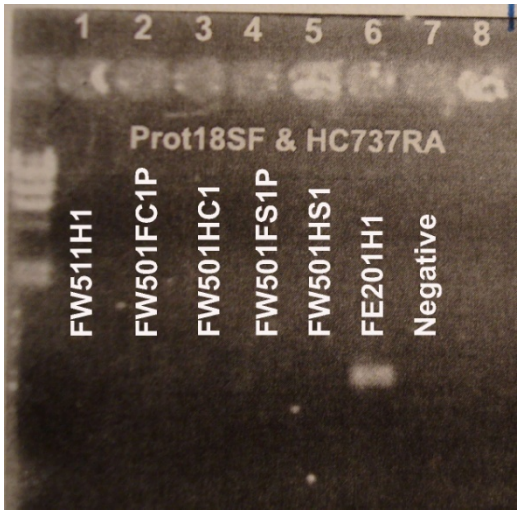


Figure 4.6 PCR with *H. carunculata*-specific primer Hc737RA. The agarose gel in this picture shows the PCR reaction using the *H. carunculata* primer (Hc737RA). The positive in the reaction was tissue sample from *H. carunculata* (FE201H1, well 6). The negative control was the protist CCMP768 (well 7). The DNA in the 5 other samples (FW511H1, FW501FC1P, FW501HC1, FW501FS1P, FW501HS1) was not amplified in the presence of the *H. carunculata*-specific primer.

Discussion

The results of this study provide a baseline of knowledge to be used in future development of diagnostic tools to distinguish predation from other agents of coral tissue loss. Since visual observations are not sufficient to diagnose a causal agent of tissue loss (Zorpette 1995, Bruckner 2006, Ainsworth et al. 2007) this approach provides information on potential analytical tools to understand the feeding behavior and mechanisms of this coral reef predator.

This study took place during the third year (2007) of consecutive winter outbreaks of apparently rapid tissue loss (aka white syndrome) in the FGBNMS. The corallivore *H. carunculata* was found consuming coral tissue at the margin of *M. annularis* colonies

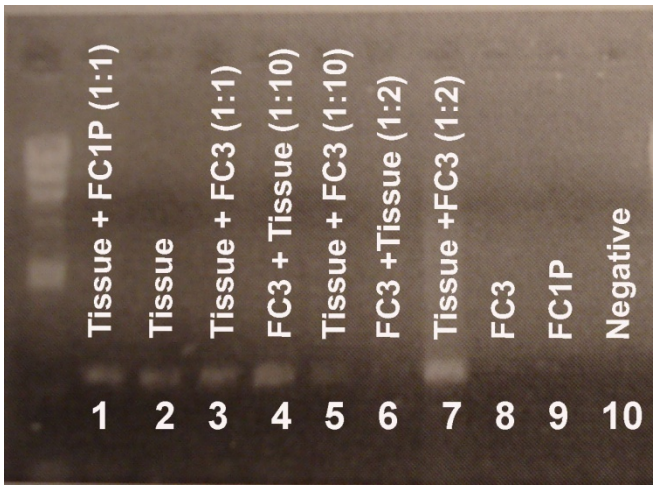


Figure 4.7 PCR with *H. carunculata*-specific primer testing for interference from coral DNA The agarose gel in this picture shows the PCR products using the *H. carunculata*-specific primer Hc737RA. The positive control was the tissue sample (well 2). The negative controls were pure coral DNA (well 8) and no DNA (well 10). Wells 1 and 3-7 had different ratios of coral DNA and *H. carunculata* DNA. Well 1 was a 1:1 ratio of *H. carunculata* DNA to coral DNA. Well 3 was a 1:10 ratio of coral DNA to *H. carunculata* DNA. Well 4 was a 1:10 ratio of *H. carunculata* DNA to coral DNA. Wells 5 and 6 had DNA ratios of 1:2 (*H. carunculata* DNA to coral DNA and coral DNA to *H. carunculata* DNA, respectively). Well 9 contained product using coral DNA (FC1P) associated with *H. carunculata* feeding. Most samples (except in well 6) with *H. carunculata* DNA were amplified in the presence of coral DNA.

suspected of having recent tissue loss due to WP Furthermore, the unusually high densities of *H. carunculata* (Lewis and Crooks 1996) feeding during peak daylight hours was not consistent with previous studies on the feeding behavior of this species (Ott and Lewis 1972). The high density of this corallivore, its proximity to the tissue loss margin, and the understanding that microbial activity is not generally active during cold water temperatures (Ben-Haim et al. 2003) suggested *H. carunculata* might be contributing to the tissue loss outbreak in the FGBNMS. It must be noted that diving operations at the FGBNMS were limited due to weather and time constraints because of the depth of the

reefs, thus it was impossible to determine whether and how tissue loss occurred prior to finding the fireworms at the tissue margin and to assess whether the tissue loss continued after the fireworms removed during this study. However, the limited information available about the predation scars of *H. carunculata* makes is difficult to identify *H. carunculata*-induced tissue loss in field observations (Miller and Williams 2007, Rotjan and Lewis 2008).

To address the question of whether *H. carunculata* feeding behavior leaves behind a molecular marker unique to the fireworm, actively feeding specimens, coral cores, and swabs were taken from coral colonies at tissue loss margins at FGBNMS in March 2007. Genomic DNA was extracted from the samples. All samples with eukaryotic DNA were run through PCRs with primers designed to be highly selective for *H. carunculata.* Of the 40 samples with eukaryotic DNA, only the three samples extracted from *H. carunculata* tissue (positive controls) were amplified by the *H. carunculata*-specific primers. Interestingly, the swabs sampled directly from the mouths of the specimens were not amplified by the specific primers. More tests were conducted with the specific primers to investigate a potential interference from coral DNA when mixed with *H. carunculata* DNA. Even in the products with the largest ratio of coral DNA (1.8 µl) to *H. carunculata* DNA (0.2 µl), *H. carunculata* DNA was amplified. These results indicate that *H. carunculata* DNA is not detectable at feeding sites through molecular analytical techniques. Therefore, the working hypothesis is rejected and the null hypothesis is accepted. *Hermodice carunculata* does not leave a molecular marker at foraging sites, at least under the conditions of this study.

The need for *H. carunculata* research is apparent given the observations of their foraging on tissue margins in the FGBNMS and Navassa during outbreaks of suspect WP. However, molecular techniques may not be effective tools to use for detecting predation sites. The *H. carunculata* DNA that might have been left at feeding sites could be affected by digestive enzymes secreted by the organism to aid in tissue extraction. Residual digestive enzymes on the coral tissue or swabs might break down, interfere, or damage some of the *H. carunculata* DNA on the sample swabs before they could be frozen or after thawing.

Another possible explanation for *H. carunculata* DNA not amplifying in this series of PCRs could be the primers. The selective primers amplified pure *H. carunculata* tissue. Future molecular studies should use a new set of primers prepared from a different region of amplification. Also, molecular techniques should include the use of Amplicon Length Heterogeneity (LH-PCR) to obtain molecular fingerprints. This approach will allow a more thorough understanding of the DNA present in the samples, including the presence of *H. carunculata* DNA.

Corallivores and their population controls have been overlooked in previous studies assessing the impact of coral tissue loss on reef ecosystems (Ott and Lewis 1972, Birkeland and Lucas 1990, Sussman et al. 2003, Dalton and Godwin 2006, Miller and Williams 2007). Extensive research is needed to understand the role of *H. carunculata* and other corallivores in coral tissue loss and the etiology of coral diseases (Rotjan and Lewis 2008). The field observations in this study and other documented coral predator events indicate corallivory may be an important part in suspect disease outbreaks and

therefore should always be considered when investigating causal agents of coral tissue loss.

Literature Cited

Ainsworth TD, Kramasky-Winter E, Loya Y, Hoegh-Guldberg O, Fine M (2007) Coral disease diagnostics: What's between a plague and a band? Appl Environ Microbiol 73:981-992

Alker AP, Smith GW, Kim K (2001) Characterization of *Aspergillus sydowii* (Thom et Church), a fungal pathogen of Caribbean sea fan corals. Hydrobiol 460:105-111

Al-Moghrabi S (2001) Unusual black band disease (BBD) outbreak in the northern tip of the Gulf of Aqaba (Jordan). Coral Reefs 19:330-331

Antonius A (1977) Coral mortality in reefs: a problem for science and management 3rd International Coral Reef Symposium, Miami, p 617-623

Aronson RB, Precht WF (2001) White-band disease and the changing face of Caribbean coral reefs. Hydrobiol 460:25-38

Bak RPM, Meesters EH (1998) Coral population structure: the hidden information of colony size-frequency distributions. Mar Ecol-Prog Ser 162:301-306

Ben-Haim Y, Rosenberg E (2002) A novel *Vibrio* sp. pathogen of the coral *Pocillopora damicornis*. Mar Biol 141:47-55

Ben-Haim Y, Zicherman-Keren M, Rosenberg E (2003) Temperature-regulated bleaching and lysis of the coral *Pocillopora damicornis* by the novel pathogen *Vibrio coralliilyticus*. Appl Environ Microbiol 69:4236-4242

Birkeland C, Lucas J (eds) (1990) *Acanthaster planci*: major management problem of coral reefs, Vol. CRC Press

Bruckner AW (2001) Coral health and mortality. Recognizing the signs of coral diseases and predators. In: Humann P, Deloach N (eds) Reef Coral Identification: Florida, Caribbean, Bahamas, Including Marine Plants, pp 240-271

Bruckner AW (2006) Progress in Understanding Coral Diseases in the Caribbean Coral Health and Disease in the Pacific: Vision for Action, Honolulu, Hawaii

Bruckner AW, Bruckner RJ (1997) Outbreak of coral disease in Puerto Rico. Coral Reefs 16:260

Bruckner AW, Bruckner RJ (2006) Consequences of yellow band disease (YBD) on *Montastraea annularis* (species complex) populations on remote reefs off Mona Island, Puerto Rico. Dis Aquat Org 69:67-73

Bruno JF, Petes LE, Harvell CD, Hettinger A (2003) Nutrient enrichment can increase the severity of coral diseases. Ecol Lett 6:1056-1061

Bruno JF, Selig ER, Casey KS, Page CA, Willis BL, Harvell CD, Sweatman H, Melendy AM (2007) Thermal stress and coral cover as drivers of coral disease outbreaks. PLoS Biol 5:1220-1227

Bythell JC, Gladfelter EH, Bythell M (1993) Chronic and catastrophic natural mortality of 3 common Caribbean reef corals. Coral Reefs 12:143-152

Bythell JC, Pantos O, Richardson LL (2004) White plague, white band, and other "white" diseases. In: Rosenberg E, Loya Y (eds) *Coral Health and Disease*. Springer-Verlag, Berlin, p 351-366

Cerrano C, Bavestrello G, Bianchi CN, Cattaneo-vietti R, Bava S, Morganti C, Morri C, Picco P, Sara G, Schiaparelli S, Siccardi A, Sponga F (2000) A catastrophic mass-mortality episode of gorgonians and other organisms in the Ligurian Sea (Northwestern Mediterranean), summer 1999. Ecol Lett 3:284-293

Coles SL, Seapy DG (1998) Ultraviolet absorbing compounds and tumorous growths on acroporid corals from Bandar Khayran, Gulf of Oman, Indian Ocean. Coral Reefs 17:195-198

Cumming RL (2002) Tissue injury predicts colony decline in reef-building corals. Mar Ecol-Prog Ser 242:131-141

Dalton SJ, Godwin S (2006) Progressive coral tissue mortality following predation by a corallivorous nudibranch (*Phestilla* sp.). Coral Reefs 25:529-529

Denner EBM, Smith GW, Busse HJ, Schumann P, Narzt T, Polson SW, Lubitz W, Richardson LL (2003) *Aurantimonas coralicida* gen. nov., sp. nov., the causative agent of white plague type II on Caribbean scleractinian corals. Int J Syst Evol Microbiol 53:1115-1122

Dustan P (1977) Vitality of reef coral populations off Key Largo, Florida: recruitment and mortality. Environ Geol 2:51-58

Edmunds PJ, Elahi R (2007) The demographics of a 15-year decline in cover of the Caribbean reef coral *Montastraea annularis*. Ecol Monogr 77:3-18

Frias-Lopez J, Zerkle AL, Bonheyo GT, Fouke BW (2002) Partitioning of bacterial communities between seawater and healthy, black band diseased, and dead coral surfaces. Appl Environ Microbiol 68:2214-2228

Gardner TA, Cote IM, Gill JA, Grant A, Watkinson AR (2003) Long-term region-wide declines in Caribbean corals. Science 301:958-960

Geiser DM, Taylor JW, Ritchie KB, Smith GW (1998) Cause of sea fan death in the West Indies. Nature 394:137-138

Gil-Agudelo DL, Garzon-Ferreira J (2001) Spatial and seasonal variation of Dark Spots Disease in coral communities of the Santa Marta area (Colombian Caribbean). Bull Mar Sci 69:619-629

Glynn PW (1963) *Hermodice carunculata* and *Mithraculus sculptus,* two hermatypic coral predators. Association of Island Marine Laboratories 4th Meeting pp 16-17

Harvell CD, Kim K, Burkholder JM, Colwell RR, Epstein PR, Grimes DJ, Hofmann EE, Lipp EK, Osterhaus A, Overstreet RM, Porter JW, Smith GW, Vasta GR (1999) Review: Marine ecology - Emerging marine diseases - Climate links and anthropogenic factors. Science 285:1505-1510

Harvell D, Jordan-Dahlgren E, Merkel S, Rosenberg E, Raymundo L, Smith G, Weil E, Willis B (2007) Coral disease, environmental drivers, and the balance between coral and microbial associates. Oceanogr 20:172-195

Henry LA, Hart M (2005) Regeneration from injury and resource allocation in sponges and corals - A review. Int Rev Hydrobiol 90:125-158

Hughes TP (1994) Catastrophes, phase shifts, and large scale degradation of a Caribbean coral reef. . Science 265:1547-1551

Hughes TP, Tanner JE (2000) Recruitment failure, life histories, and long-term decline of Caribbean corals. Ecology 81:2250-2263

Israely T, Banin E, Rosenberg E (2001) Growth, differentiation and death of *Vibrio shiloi* in coral tissue as a function of seawater temperature. Aquat Microb Ecol 24:1-8

Kim K, Harvell C (2002) Aspergillosis of sea fan corals: disease dynamics in the Florida Keys. In: Porter J, Porter K (eds) The Everglades Florida Bay and coral reefs of the Florida Keys: an ecosystem sourcebook. CRC Press, New York, p 813-824

Knowlton N (2001) The future of coral reefs. Natl Acad Sci, pp 5419-5425

Kramarsky-Winter E, Loya Y (2000) Tissue regeneration in the coral *Fungia granulosa*: the effect of extrinsic and intrinsic factors. Mar Biol 137:867-873

Kushmaro A, Rosenberg E, Fine M, Loya Y (1997) Bleaching of the coral *Oculina patagonica* by *Vibrio* AK-1. Mar Ecol-Prog Ser 147:159-165

Kuta KG, Richardson LL (2002) Ecological aspects of black band disease of corals: relationships between disease incidence and environmental factors. Coral Reefs 21:393-398

Lewis JB, Crooks RE (1996) Foraging cycles of the amphinomid polychaete *Hermodice carunculata* preying on the calcareous hydrozoan *Millepora complanata.* Bull Mar Sci 58:853-856

Lizama J, Blanquet RS (1975) Predation on sea anemones by the amphinomid polychaete, *Hermodice carunculata*. Bull Mar Sci 25:442-443

Luna GM, Biavasco F, Danovaro R (2007) Bacteria associated with the rapid tissue necrosis of stony corals. Environ Microbiol 9:1851-1857

Marsden JR (1962) A coral-eating polychaete. Nature 193:598

Marsden JR (1963) The digestive tract of *Hermodice carunculata.* (Pallas). Polychaeta: Amphinomidae. Can J Zool/Rev Can Zool 41:165-184

Martin A, Losada F (1991) Regeneration of injuries caused by the amphinomid polychaete *Hermodice carunculata* on scleractinian corals, hydrocorals and octocorals (Cnidaria). Ecotropicos 4:18-26

Miller MW, Williams DE (2007) Coral disease outbreak at Navassa, a remote Caribbean island. Coral Reefs 26:97-101

Nagelkerken I, Bak RPM (1998) Differential regeneration of artificial lesions among sympatric morphs of the Caribbean corals *Porites astreoides* and *Stephanocoenia michelinii*. Mar Ecol-Prog Ser 163:279-283

Nagelkerken I, Meesters EH, Bak RPM (1999) Depth-related variation in regeneration of artificial lesions in the Caribbean corals *Porites astreoides* and *Stephanocoenia michelinii*. J Exp Mar Biol Ecol 234:29-39

Ott B, Lewis JB (1972) The importance of the gastropod *Coralliophila abbreviata* (Lamarck) and the polychaete *Hermodice carunculata* (Pallas) as coral reef predators. Can J Zool/Rev Can Zool 50:1651-1656

Pandolfi JM, Bradbury RH, Sala E, Hughes TP, Bjorndal KA, Cooke RG, McArdle D, McClenachan L, Newman MJH, Paredes G, Warner RR, Jackson JBC (2003) Global trajectories of the long-term decline of coral reef ecosystems. Science

301:955-958

Pantos O, Cooney RP, Le Tissier MDA, Barer MR, O'Donnell AG, Bythell JC (2003) The bacterial ecology of a plague-like disease affecting the Caribbean coral *Montastraea annularis*. Environ Microbiol 5:370-382

Patterson KL, Porter JW, Ritchie KB, Polson SW, Mueller E, Peters EC, Santavy DL, Smith GW (2002) The etiology of white pox, a lethal disease of the Caribbean elkhorn coral, *Acropora palmata*. Nat Acad Sci 99:8725-8730

Porter JW, Dustan P, Japp WC, Patterson KL, Kosmynin V, Meier OW, Patterson ME, Parsons M (2001) Patterns and spread of coral disease in the Florida Keys. Hydrobiol. 460:1-24

Raymundo LJ, Rosell KB, Reboton CT, Kaczmarsky L (2005) Coral diseases on Philippine reefs: genus *Porites* is a dominant host. Dis Aquat Org 64:181-191

Remily ER, Richardson LL (2006) Ecological physiology of a coral pathogen and the coral reef environment. Microb Ecol 51:345-352

Richardson LL (1998a) Coral diseases: what is really known? Trends Ecol Evol 13:438-443

Richardson LL, Goldberg WM, Carlton RG, Halas JC (1998b) Coral disease outbreak in the Florida Keys: Plague type II. Rev Biolog Tropical 46:187-198

Richardson LL, Goldberg WM, Kuta KG, Aronson RB, Smith GW, Ritchie KB, Halas JC, Feingold JS, Miller SL (1998c) Florida's mystery coral-killer identified. Nature 392:557- 558

Richardson LL, Kuta KG (2003) Ecological physiology of the black band disease cyanobacterium *Phormidium corallyticum*. FEMS Microbiol Ecol 43:287-298

Riegl B (2002) Effects of the 1996 and 1998 positive sea-surface temperature anomalies on corals, coral diseases and fish in the Arabian Gulf (Dubai, UAE). Mar Biol 140:29-40

Ritchie KB, Smith GW (1998) Type II white-band disease. Revista De Biologia Tropical 46:199-203

Rosenberg E, Ben-Haim Y, Toren A, Banin E, Kushmaro A, Fine M, Loya Y (1998) Effect of temperature on bacterial bleaching of corals. In: Rosenberg E (ed) Current perspectives in microbial ecology. ASM Press, Washington, D.C., pp 242-254

Rosenberg E, Loya Y (2004) *Coral Health and Disease*, Vol. Springer-Verlag, Berlin

Rotjan RD, Lewis SM (2005) Selective predation by parrotfishes on the reef coral *Porites astreoides*. Mar Ecol-Prog Ser 305:193-201

Rotjan RD, Lewis SM (2008) Impact of coral predators on tropical reefs. Mar Ecol-Prog Ser 367:73-91

Shinn EA, Smith GW, Prospero JM, Betzer P, Hayes ML, Garrison V, Barber RT (2000) African dust and the demise of Caribbean coral reefs. Geophys Res Lett 27:3029-3032

Smith G, Weil E (2004) Aspergillosis in gorgonians. In: Rosenberg E, Loya Y (eds) *Coral Health and Disease*. Springer Verlag, pp 279-288

Sussman M, Loya Y, Fine M, Rosenberg E (2003) The marine fireworm *Hermodice carunculata* is a winter reservoir and spring-summer vector for the coral-bleaching pathogen *Vibrio shiloi*. Environ Microbiol 5(4):250-255

Sutherland KP, Porter JW, Torres C (2004) Disease and immunity in Caribbean and Indo-Pacific zooxanthellate corals. Mar Ecol Prog Ser 266:273-302

Vogler C, Benzie J, Lessios H, Barber P, Worheide G (2008) A threat to coral reefs multiplied? Four species of crown-of-thorns starfish. Biol Lett 4:696-699

Weil E, Croquer A (2009) Spatial variability in distribution and prevalence of Caribbean scleractinian coral and octocoral diseases. I. Community-level analysis. Dis Aquat Org 83:195-208

Weil E, Smith G, Gil-Agudelo DL (2006) Status and progress in coral reef disease research. Dis Aquat Org 69:1-7

Weil E, Urreiztieta I, Garzón-Ferreira J (2002) Geographic variability in the incidence of coral and octocoral diseases in the wider Caribbean. 9th International Coral Reef Symposium, Bali, Indonesia, pp 1231–1237

Wilkinson C (ed) (2004) Status of coral reefs of the world, Vol 1. Global Coral Reef Monitoring Network and Australian Institute of Marine Science, Townesville, Australia

Williams DE, Miller MW (2005) Coral disease outbreak: pattern, prevalence and transmission in *Acropora cervicornis*. Mar Ecol-Prog Ser 301:119-128

Witman JD (1988) Effects of predation by the fireworm *Hermodice carunculata* on Milleporid hydrocorals. Bull Mar Sci 42:446-458

Zorpette G (1995) More coral trouble. Sci Am 273:36-37

5. Overall Conclusions

1. Gut content varied in *Hermodice carunculata* specimens. The most common materials were collagenous cuticle and coccoid cells. In Chapter 3, *H. carunculata* specimens lost weight while feeding on *Millepora complanata.* These results suggest *H. carunculata* obtains a sustainable nutritional value from consuming prey in addition to coral tissue. A reduction in live coral tissue may not have a direct impact on *H. carunculata* survivorship but may increase *H. carunculata*'s foraging time on alternative prey. Also, the availability of alternative prey may be influenced by changes in physical and biological structures of coral reefs which may also affect *H. carunculata* nutritional intake. Research is needed on *H. carunculata* species' diet composition, diet requirements, and the nutritional value of its food to qualify the direct and indirect impacts of coral tissue loss on *H. carunculata* and its prey.

2. Through histological observations, a layer of coccoid cells was found on the outer cuticle and a several clusters within the digestive tract and foregut of *H. carunculata*. Also, *H. carunculata* was associated with tissue loss margins of suspect white plague in the Flower Garden Banks National Marine Sanctuary. Bacteria associated with this organism may be transmitted during movement and/or during foraging activities. A layer of bacteria on the outer cuticle may serve as protection from potentially harmful

microbes or other organisms. This layer of cells may also provide opportunities for microbes to attach to *H. carunculata* organisms for transportation to other coral colonies. Bacteria in the foregut may be transmitted when *H. carunculata* everts its pharynx during feeding which, if pathogenic, could exacerbate tissue loss or induce disease. Bacteria staining and microscopic analysis in needed to determine if the coccoid cells on the outer cuticle and with the digestive system are bacteria. Field observations are needed at foraged sites to see if tissue margin continues after a predation episode. More research on the ability to uptake and distribute bacteria either from the outer cuticle or during feeding is needed to understand the vector capabilities of this species.

3. *H. carunculata* has primitive eyes, a dorsal nuchal organ, and a ventral nerve cord which may have sensory functions to find prey. These features may pick up environmental cues as chemoreceptors and photoreceptors. *H. carunculata* has feeding features including digestives enzymes in the muscular proboscis and a flexible and expandable buccal region which help to consume prey of various sizes and types. The ability to eat various prey is an important adaptative characteristic for inhabitants of a changing environment like coral reefs. Digestive enzymes may help consume prey through breakdown and removal of tissue or materials from various substrate. The ventral cord may interact with the substrate through pigmented cells. Observations and studies examining the function the sensory features are needed to

understand how *H. carunculata* receives environmental cues to find prey and avoid predators.

4. *H. carunculata* has various defense mechanisms. Tufts of setae will become brighter in color when an object is near the organism. The setae are toxic and they break off or are released when *H. carunculata* is handled. Histological observations confirm field observations on the different coloration patterns of this species. Individuals can be various patterns of green, red, yellow, and brown. The color schemes may help the organism blend into the substrate to disguise from potential predators. Though specific predators have not been identified in literature or observations, the presence of biological structures which deter predators suggests there is an evolutionary pressure to develop these features. A driving force for defense mechanism may be *H. carunculata*'s feeding behavior. Its pharynx is everted and the organism is stationary on the reef surface during the predation episode. Therefore, it may be vulnerable to predators when consuming coral tissue and moving along the coral substrate in search of prey.

5. *H. carunculata* is a poorly understood coral predator and reef dweller. Histological studies in Chapter 2 found new features which may have ecological implications. Results from the feeding experiments in Chapter 3 suggest coral tissue does not provide enough nutrients for survivorship. Field observations on *H. carunculata* feeding behavior associated with tissue loss margins of suspect-WP and the presence

of coccoid cells resembling bacteria in the body of *H. carunculata* suggests this species has vector capabilities. This organism and its role in coral tissue loss through feeding and transmission of pathogenic bacteria need to be well understood. Documentation of predation scars and patterns are needed to distinguish *H. carunculata*-induced lesions. Also, questions about the vulnerability of coral colonies after predation episodes to future foraging by the same organism or other corallivores or the vulnerability of coral tissue to pathogenic microbes after predation need to be answered to determine this species' contribution to overall coral tissue loss.

CURRICULUM VITAE

Staci Lewis received her Bachelors of Science degree from Salem College in 2002. She started studing *Hermodice carunculata* in Barbados as a research assistant under Dr. Suzanne Dorsey. From 2003-2005, she worked as the laboratory and projects manager for Dr. Rich Aronson at the Dauphin Island Sea Lab in Alabama and worked on various coral reef research project, She conducted studies on *Hermodice carunculata* during her Fulbright Fellowship in Barbados from 2005-2006.