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Carcinonemertes conanobrieni - A Nemertean Parasite Infecting the Caribbean Spiny Lobster, *Panulirus argus*. Species Description, Host-Use, and Effect on Host Reproductive Health

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CARCINONEMERTES CONANOBRIENI – A NEMERTEAN PARASITE INFECTING THE CARIBBEAN SPINY LOBSTER, *PANULIRUS ARGUS*. SPECIES DESCRIPTION, HOST-USE, AND EFFECT ON HOST REPRODUCTIVE HEALTH

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Abstract

Marine ecosystems are one of the world's most heavily used and valuable natural systems. However, over the past decades, they have seen changes in the oceans' pH, temperature, salinity, and other abiotic factors - all of which appear to have impacted the health of these systems, and there seems to be a global trend indicating that diseases in marine environments are emerging at an increased rate. Infection by a disease can result in a variety of negative effects on the health of a host, all of which are especially relevant in instances where commercially important hosts are infected. Disease can lead to changes in growth, longevity, reproduction, embryo survival, and marketability of a host. One ecologically and commercially important species that appears to have been impacted by this trend of increased disease emergence is the Caribbean spiny lobster, *Panulirus argus*. *Panulirus argus* plays host to a number of previously described and newly emergent pathogens. However, here, a new species of nemertean worm belonging to the genus *Carcinonemertes* is described from egg masses of *P. argus* from the Florida Keys, Florida, USA. Though *P. argus* ranges throughout the Caribbean, this worm has thus far only been observed infecting gravid female lobsters in the Florida Keys. This is the first species of *Carcinonemertes* reported to infect *P. argus* or any other lobster species in the greater Caribbean and western Atlantic Ocean. To determine the host use, infection prevalence, and infection intensity of this new parasite on *P. argus*, male, non-gravid female, and gravid female lobsters were captured along the Florida Key reef tract from and examined for infection. Furthermore, infected gravid females were also used in

estimating the impact that infection by this nemertean had on three levels of reproductive performance (reproductive output, fecundity, and brood mortality).

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Chapter 1

An Introduction

Background

Marine ecosystems are one of the world's most heavily used and valuable natural systems, and they provide important ecosystem services - including supporting fishing, food, and pharmaceutical industries, CO_2 absorption, water filtration, shoreline protection, tourism services, and others (Suttle, 2007; Hoegh-Guldberg & Bruno, 2010; Staudinger et al., 2013; Ruckelshaus et al., 2013). However, over the past decades global climate change and human disturbances have led to changes in the oceans' pH, temperature, salinity, and other abiotic factors (Gilman et al., 2008). It has been demonstrated that these types of changes have the ability to effect the survival, growth, and health of marine organisms (Doney et al., 2012). And these changes appear to have played a role in recent mass mortalities of fish, coral, sponge, and other invertebrate communities (Harvell et al., 1999; Hayes et al., 2001; Lafferty et al., 2004; Ward & Lafferty, 2004). These mortalities, and the probable factors driving them, have led to a global increase in research focusing on the health of the oceans, and specifically in occurrences of disease outbreak (Harvell et al., 1999; Hayes et al., 2001; Lafferty et al., 2004; Ward & Lafferty, 2004). In general, there appears to be a global trend indicating that diseases in marine environments are appearing at an increased rate (Ward & Lafferty, 2004; Lafferty, 2004). Even so, there are specific areas that are considered disease "hotspots" and are characterized by the emergence of new and more virulent diseases at an even higher rate and prevalence than other regions (reviewed in Harvell et al., 2007).

One such affected area, the Wider Caribbean, has been experiencing significant warming of its waters over the past 25 years (Chollet et al., 2012). This increase in

temperature has coincided with coral bleaching events, disease emergence, and an increasing frequency of infectious disease outbreaks (Weil et al., 2009; Eakin et al., 2010; Ruiz-Moreno et al., 2012; Burge et al., 2013). In general, infection by a disease can result in a variety of negative effects on the health of a host, all of which are especially relevant in instances where commercially important hosts are infected. Disease can lead to changes in growth, longevity, reproduction, embryo survival, and marketability of a host (Kuris et al., 1991). One ecologically and commercially important species that appears to have been impacted by this trend of increased disease emergence is the Caribbean spiny lobster, *Panulirus argus*.

Panulirus argus is a large marine invertebrate that has historically played a keystone role in its habitat, influencing overall ecosystem structure, dynamics, and function (Eddy et al., 2014). Spiny lobsters manage community structure and interactions through the direct consumption of many different benthic organisms as well as through playing prey to numerous species of higher predator (Phillips et al., 2014; Briones-Fourzan, 2015). As secondary consumers of mollusks, holothuroideans, and crustaceans they are able to make use of energy taken from their prey. Furthermore, through the consumption of bivalve mollusks, they are also able to make use of the energy produced by chemosynthetic primary producers living as symbionts of the mollusks (Higgs et al., 2016). By being highly mobile, they are able to transport this energy from one community to another, influencing the dynamics and energy availability in multiple communities (Behringer & Butler, 2006). *Panulirus argus* also plays an indirect role in

community structure through the reduction of potential predators (either as the predator or the prey) for a number of marine invertebrates (Eddy et al., 2014).

Furthermore, *P. agus* supports one of the most important fisheries in the Greater Caribbean and Gulf of Mexico area. This multimillion-dollar fishery is classified as ranging from fully-exploited to over-exploited across the entirety of its range with approximately 34,574 tons landed in 2014 (FAO fact sheet, 2017). In Florida alone, the spiny lobster trap fishery represents 91% of commercial landings for the state (Buesa, 2018). However, over the past decades, the spiny lobster commercial landings have been on the decline (approx. 30%) and are well below historical figures (Ehrhardt et al., 2010; Behringer et al., 2012). The commercial importance of this fishery and its steady decline over the years has resulted in a large and varied body of literature covering the anatomy, physiology, behavior, ecology, and life history of *P. argus* (for review: Holthuis, 1991).

The life history of *P. argus* is complex, with planktotrophic larvae spending anywhere from 4 to 18 months suspended in the water column, before migrating to inshore habitats and settling in seagrass or macro-algal beds, where they then molt into a first stage juveniles (Butler and Herrnkind, 2000; Phillips et al., 2007; Espinosa-Magana, 2017). Juvenile and sub-adult lobsters are then attracted to the cues of conspecifics, and may be found inhabiting shared dens (Childress and Herrnkind, 1996; 1997). Because of this aggregate behavior, there is the potential for infectious diseases to spread rapidly through a population of lobsters (Butler et al., 2015). And while lobsters may be infected by a pathogen at any life stage, juveniles have been shown to be the targets of some of the most pervasive and significant diseases (Behringer, 2012). Research into these

diseases and their effects on *P. argus* has shown that while the lobster plays host to a variety of marine diseases and pathogens, very few have lethal effects (reviewed in: Shields et al., 2006; Shields, 2011).

One such lethal pathogen that has the high potential to impact fishery management practices is *Panulirus argus* Virus 1 (PaV1) (Shields & Behringer, 2004). PaV1 has been demonstrated as being nearly 100% lethal to sub-adults and juvenile lobsters that contract the virus (Shields & Behringer, 2004). Some non-lethal pathogens that have been found to infect *P. argus* include multiple species of bacteria (*Aerococcus viridans* – Bobes et al., 1988; *Vibrio* spp. – Silva dos Fernandes Vieira et al., 1987; and other genera – Porter et al., 2001), helminths (*Cymatocarpus solearis* – Gomez del Prado-Rosas et al., 2003), and crustaceans (*Balanomorphs* – Eldred, 1962). While *Carcinonemertes* worms have been found to infect other spiny lobsters (*Panulirus interruptus* infected by *C. wickhami* (Shields and Kuris, 1990); *Panulirus cygnus* infected by *Carcinonemertes australiensis* (Campbell et al., 1989)) as of yet, there have been no reports of a *Carcinonemertes* species worm infecting *P. argus*.

Carcinonemertes is one of two genera that comprises the family Carcinonemertidae within the phylum Nemertea (the other being *Ovicides*) (Giribet et al., 2009). All members of the family are considered specialized parasites of decapod crustaceans that consume the embryos of their gravid hosts (Shields, 2001). To date, there are 16 described species of *Carcinonemertes*, and 5 described species of *Ovicides* found in association with approximately 70-75 recorded host species (Humes, 1942; Wickham and Kuris, 1985; Shields and Segonzac, 2007; Sadeghian and Santos, 2010) with most

occurring on cancrid, portunid, and xanthid crabs; though as mentioned above, two (*C. australiensis* and *C.wickhami*) have been found infecting palinurid lobsters. Members of this nemertean family vary in terms of host specificity, with some species (*C. errans* - Wickham, 1978; and *O. juliaea* - Shields, 2001) inhabiting a single host species while others (*C.c. carcinophila* and *C.c. imminuta*) are reported on more than a dozen decapod species of crab (Humes, 1942; Shields and Segonzac, 2007). These worms are often overlooked because they usually show low prevalence in host populations, they live in cryptic locations on the host, and/or they typically only mature on ovigerous hosts, meaning in some instances they may be observed only seasonally (Shields, 1992; Shields and Segonzac, 2007). And while many of these worm species exhibit low background infection and embryo loss of the host is usually around 5% (Wickham, 1980; Wickham and Kuris, 1985) years of epidemic levels of infection have led to 100% embryo loss and resulted in no new recruitment for the following year (Wickham, 1980; Wickham and Kuris, 1985).

During an investigation into the active parental care and reproductive performance of *P. argus* in the Florida Keys, I discovered the presence of a parasitic worm in the broods of gravid female lobsters. Preliminary data reported in Baeza et al. (2016) suggested that the parasite impacts reproductive performance for infected brooding females. Because *P. argus* is such an important fishery resource in the wider Caribbean, detailing the relationship and the impact that this worm has on such a commercially valuable host is an important step in describing the condition of the spiny lobster fishery in the Florida Keys, and perhaps finding another factor behind the

declining lobster landings. Furthermore, as a keystone species in the wider Caribbean, lobsters hold an important role in predator/prey dynamics as well as competitive interactions (Behringer et al., 2012). By understanding how this worm impacts *P. argus* I can improve our understanding of future changes to community interactions and population dynamics.

Using the discovery of the nemertean, and initial observations I will describe this new species of *P. argus* parasite, and then describe the effects that infection by this worm has on the host. To accomplish this, I will begin with a morphological and genetic description of this new parasite. Following, I will describe the morphology and development of the nemertean larvae. I will then describe the host-use pattern of the parasite on *P. argus* and determine if it infects both sexes and all life-stages of its host, as well as determine the prevalence and intensity of infection. And finally, I will investigate the impact that this parasitic nemertean has on the reproductive performance of infected hosts by comparing the reproductive output, fecundity, and brood mortality of infected and non-infected brooding females.

In the second chapter of this thesis, I test that this nemertean is a new species using 23 morphological characters and COI sequences to compare against all previously described *Carcinonemertes* species, *Ovicides* species, and other closely related nemerteans. I propose that this worm will exhibit enough morphological and genetic differences to confirm that it is a new species. Furthermore, I believe that the results of the genetic analysis will show that this worm has close relatedness to other *Carcinonemertes* species worms when it is compared to both *Ovicides* and the outgroup

species, confirming its position in the genus. In the third chapter of this thesis, I describe larval morphology and development of *C. conanobrieni*. I use high resolution confocal images of 0 day, 5 day, and 10 day old larvae to build a time-line of neural and muscular development, and make comparisons across this span. I also make note of distinct behaviors of adult worms, including feeding, mating, embryo release, and others, that can be used to help determine the identity of this worm in future studies. In the fourth chapter of this thesis, I investigate the host-use of the nemertean and the effect that it has on the reproductive performance of brooding female lobsters. I propose, that since this nemertean likely belongs to the genus *Carcinonemertes* that it will be found disproportionally on gravid female lobsters with infection concentrated on the brood mass, when compared to male and non-brooding females. Furthermore, as *Carcinonemertes* species worms are egg predators, the worms found on *P. argus* all likely consume the embryos of their hosts, and will have a negative impact on their reproductive performance through a decrease in fecundity and reproductive output and an increase in brood mortality.

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Chapter 2

A New Species of *Carcinonemertes*, *Carcinonemertes conanobrieni* sp. nov. (Nemertea: Carcinonemertidae), an Egg Predator of the Caribbean Spiny Lobster, *Panulirus argus*

Abstract

A new species of nemertean worm belonging to the genus *Carcinonemertes* is described from egg masses of the Caribbean spiny lobster *Panulirus argus* from the Florida Keys, Florida, USA. Though *P. argus* ranges throughout the Caribbean, this worm has thus far only been observed infecting gravid female lobsters in the Florida Keys. This is the first species of *Carcinonemertes* reported to infect *P. argus* or any other lobster species in the greater Caribbean and western Atlantic Ocean. *Carcinonemertes conanobrieni* sp. nov. varies in body color from a translucent white to a pale orange, with males ranging in total body length from 2.35 to 12.71 mm and females ranging from 0.292 to 16.73 mm. Among the traits that separate this new species from previously described species in genus *Carcinonemertes* are a relatively wide stylet basis, minimal sexual size dimorphism, and a unique mucus sheath decorated with external hooks. Also, juvenile worms were found to encyst themselves next to lobster embryos and female worms lay both long strings of eggs wound throughout the lobster's setae as well as spherical cases that are attached to lobster embryos. The stylet length and stylet basis remain unchanged throughout ontogeny for both male and female worms. Maximum likelihood and Bayesian inference phylogenetic analyses separated this newly described species from all other species of *Carcinonemertes* with available COI sequences. *Carcinonemertes* spp.are voracious egg predators and have been tied to the collapse of various crustacean fisheries. The formal description of this new species represents the first step to understand putative impacts of this worm on the population health of one of the most lucrative yet already depressed crustacean fisheries.

Introduction

Marine ecosystems are one of the most heavily used and valuable natural systems worldwide [1] providing globally relevant ecosystem services (e.g., shoreline protection, water filtration, nursery grounds, feeding grounds to commercially important fishes – [2, 3]). At the same time, these complex and well-interconnected marine systems are vulnerable to both natural and human perturbations [4]. Global climate change and increasing ocean temperatures, among others, have been shown to impact the survival, growth, and health of marine organisms [5] and periods of thermal stress have led to disease outbreaks [6, 7]. As ocean temperature rises many marine organisms, including pathogens, are shifting towards the poles [8, 9] leading to changes in the interactions between hosts and pathogens. This in turn, has the potential to lead to changes in the frequency and severity of disease events [7; reviewed in 3]. One such affected area, the wider Caribbean region, is considered a disease hot spot characterized by the rapid emergence of a variety of new and virulent diseases, and typically at a higher prevalence than in other regions [reviewed in 10]. Over the past 25 years the significant warming of the Caribbean basin [11] has coincided with coral bleaching events, disease emergence, and an increasing frequency of infectious disease outbreaks [12-14; 3].

Spiny lobsters, including the Caribbean spiny lobster, *Panulirus argus*, have been shown to play host to a variety of marine diseases and pathogens, including some newly emergent diseases [reviewed in 15, 16]. *Panulirus argus* Virus 1 (PaV1) is one such emergent disease infecting *P. argus* and was first reported in 2004 by Shields and Behringer [17]. *Panulirus argus* is also know to host multiple species of bacteria [18-20],

helminths [21], and crustaceans [22]. Though *P. argus* has not yet been reported to host a *Carcinonemertes* sp. worm, other spiny lobsters have. Examples of spiny lobster species that are infected by a *Carcinonemertes* species include: *Panulirus interruptus* (infected by *Carcinonemertes wickhami*) [23], *Panulirus cygnus* (infected by *Carcinonemertes australiensis*) [24], and *Jasus edwardsii* [16].

Carcinonemertes worms belong to the nemertean worm family Carcinonemertidae which also includes the genus *Ovicides*. Members of *Carcinonemertes* may be separated from *Ovicides* in that they possess only a single stylet with no accessory pouches and are gonochoric, while *Ovicides* is distinguished by accessory stylets and species can be either gonochoric or hermaphroditic [25, 26]. Members of this family are considered symbiotic egg predators of decapod crustaceans. To date, there are 16 described species of *Carcinonemertes*, and 5 described species of *Ovicides* found in association with approximately 70-75 recorded host species [26-29] with most occurring on cancrid, portunid, and xanthid crabs; though two species have been reported on panulirid lobsters [24, 25]. Members of this family vary in terms of host specificity, with some species inhabiting a single host species (*C. errans* [30]; and *O. juliaea* [25]) while others are reported on more than a dozen decapod species of crab (*C. c. carcinophila* and *C. c. imminuta*) [26, 27]. These worms are often overlooked because they usually show low prevalence in host populations, they live in cryptic locations on the host bodies, and/or they typically only mature on ovigerous hosts, meaning in some instances they may be observed only seasonally [26, 31].

During an investigation into the active parental care and reproductive performance of the Caribbean spiny lobster, *Panulirus argus*, in the summer of 2015, I noticed the presence of a nemertean worm in the egg mass of a few female lobsters [32]. Upon further inspection, I concluded that this nemertean belonged to the genus *Carcinonemertes*. Here I describe *Carcinonemertes conanobrieni*, a new species in the family Carcinonemertidae found in the broods of *P. argus*. Distinctive morphological characters and some aspects of the life history of this new species are discussed and presented against those of other members within the genus.

Material and Methods

Collection of host and parasite specimens

Caribbean spiny lobsters, *Panulirus argus*, were collected from July 10th to July 19th, 2016 from two coral reefs (5 - 10 m depth) along the Florida reef tract. Collection was possible through a Special Activity License through the Florida Fish and Wildlife Conservation Commission (SAL-15-1674A-SR). The first collection site was approximately 5 km off of Long Key, Florida at Tennessee Lighthouse Reef (24.7707 N, -80.7615 W) and the second site was approximately 5 km off of Duck Key, Florida at Critter Ridge Reef (24.7325 N, -80.9121 W). At each locality, gravid female lobsters were gently captured by hand (with the aid of a tickle stick and hand net) while SCUBA diving, and then transported alive in the R/V *Soledad* to a temporal laboratory in Long Key, Florida. Lobsters were maintained alive in two 416.5 liter cattle tanks with bubbling aerators until dissection.

Next, pleopods were removed from gravid females, and all embryos were gently stripped away from the pleopods and placed into Petri dishes filled with seawater using microforceps. The embryo masses were then inspected for the presence of nemerteans under either a Leica S8AP0 stereoscope or a Wild M5-97874 dissecting scope. The remainder of the host lobster anatomy (including abdomen, pleopods, eye orbitals the joints of walking legs, gills and branchial chamber) was also visually inspected to determine the presence of nemerteans using the same stereoscopes.

Nemerteans collected from lobsters were placed in Petri dishes filled with seawater until the moment of taking measurements, photographs, and notes on morphological characters. Nemerteans were first relaxed in a 1:1 solution of 1M $MgCl_2$ (prepared with distilled water) and seawater for 1-5 min., after which, length and width of the body, the distance between the eyes, and the distance from the eyes to the tip of the head were determined with the help of a micrometer slide, Leica S8AP0 Stereoscope, and Leica camera MC170 HD. Measurements of internal features were made with the help of an ocular micrometer in a compound microscope after covering the worms with a coverslip. The holotype and paratype specimens were preserved in a 7% formalin-seawater solution. Other specimens were fixed in 99% EtOH solution for genetic analysis.

Phylogenetic position of the new species

Total genomic DNA was extracted from whole specimens of the nemertean worm using the QIAGEN® DNeasy® Blood and Tissue Kit following the protocol

recommended by the manufacturer. The polymerase chain reaction (PCR) was used to amplify the target region of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene. For the amplification of COI, I used the primers LCO1490 (5'-ggt caa caa atc ata aag ata ttg g-3') and HCO2198 (5'-taa act tca ggg tga cca aaa aat ca-3') [33]. Standard PCR 25-µl reactions (12.5-µl GoTaq® MasterMix (Promega), 2.5-µl each of the two primers, and 7.5-µl of DNA template) were performed on a C1000 Touch™ Thermal Cycler (BIORAD, Hercules, CA, USA) under the following conditions: initial denaturation at 95 ºC for five minutes followed by 35 cycles of 95 ºC for 1 min, 51 ºC for 1 min, and 72 ºC for 1 min, followed by chain extension at 72 ºC for 10 minutes. The post-PCR products were purified with ExoSapIT (a mixture of exonuclease and shrimp alkali phosphate, Amersham Pharmacia) and then sent for Sanger Sequencing to Clemson University's Genomics Institute (CUGI – Clemson University, Clemson, South Carolina), which is equipped with an ABI Prism 3730xl Genetic Analyzer (Applied Biosystems automated sequencer). All sequences were confirmed by sequencing both strands and a consensus sequence was obtained from the two strands using the software Sequencer (Gene Codes Corp.).

A total of 9 other species of *Carcinonemertes* were used as ingroup terminals for molecular comparisons with the new species, with 4 other species of ribbon worm, *Ovicides* sp., *Nipponnemertes punctatula*, *Nipponnemertes bimaculata*, and *Nipponnemertes pulchra* included in the analysis as outgroup terminals. The species of *Carcinonemertes* above were chosen as they represented the totality of COI sequence data available. *Ovicides paralithodis* was chosen as an outgroup terminal because it is the

only other genera that belongs to the family Carcinonemertidae, and *Nipponnemertes bimactulata*, *Nipponnemertes punctatula* and *Nipponnemertes pulchra* were chosen as outgroup species based on recent phylogenetic studies that placed *Nipponnemertes* as sister to *Carcinonemertes* in the clade monostilifera [34]. All COI sequences, outside the ones generated by us, were retrieved from Genbank.

Sequence alignment was conducted using Multiple Sequence Comparison by Log-Expectation in MUSCLE [35] as implemented in MEGA 6 [36]. The alignment of the COI gene fragment had no indels and was unambiguous.

The dataset was first analyzed with the software jModelTest2 [37] which compares different models of DNA substitution in a hierarchical hypothesis-testing framework to select a base substitution model that best fits the given data [38]. The optimal model found by jModelTest2 (GTR for both) were implemented in MrBayes [39] for Bayesian Inference (BI) analysis and in PhyML for maximum likelihood (ML) analysis (PhyML may be accessed with: http://www.atgc-montpellier.fr/phyml/) [40]. Missing data were designated as a "?" in the alignment.

All the parameters used for the ML analysis were those of the default options in PhyML. For BI, unique random starting trees were used in the Metropolis-coupled Markov Monte Carlo Chain (MCMC) [see 39, 41]. This analysis was performed for 6,000,000 tree generations. Visual analysis of the log-likelihood scores against the generation time indicated that the log-likelihood values reached a stable equilibrium before the 100,000th generation. Thus, a burn-in of 1,000 samples was conducted and every 100th tree was sampled from the MCMC analysis obtaining a total of 60,000 trees

and a consensus tree with the 50% majority rule was calculated for the last 59,900 sampled trees. The robustness of the ML tree topology was assessed by 2,000 bootstrap iterations of the data. Support nodes for the BI tree topology were obtained by posterior probability.

Correlation Analyses

I performed classical correlation analyses between stylet characteristics (stylet length, basis length, and stylet:basis ratio) and maximum body length for both male and female *C. conanobrieni* using JMP Pro 12 Software [42]. I also used JMP Pro 12 software [42] in ANCOVA analyses that compared the stylet structures between the sexes. In these ANCOVAs, maximum body size was the independent variable, the stylet structure measurement was the dependent variable, and worm sex was set as the covariate.

Nomenclatural Acts

The electronic edition of this article conforms to the requirements of the amended International Code of Zoological Nomenclature, and hence the new names contained herein are available under that Code from the electronic edition of this article. This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information viewed through any standard web browser by appending the LSID to the prefix “http://zoobank.org/”. The LSID for

this publication is: urn:lsid:zoobank.org:pub:9D73818B-E952-4494-BF6F-4AF4FF38C7E4. The electronic edition of this work was published in a journal with an ISSN, and has been archived and is available from the following digital repositories: PubMed Central, LOCKSS.

Results

Diagnosis - Family Carcinonemertidae Sumner et al., 1913

The following diagnosis of the family Carcinonemertidae is taken from Humes [27] and modified by Shields et al. [43]: Members are monostiliferous hoplonemerteans living as symbiotic egg predators on the gills, beneath the abdomen, on the apodemes and axillae, and in the egg masses of decapod crustaceans. They possess a reduced proboscis and a short, poorly developed rhynchocoel. The lateral nerves lie internal to well-developed submuscular glands. Cephalic glands well developed, with cephalic muscle fibers present. Missing cerebral sensory organs, and possess 2 ocelli. Takakura's duct system is present in males. Internal fertilization and ovovivparity commonly occur; extensive development of spermatozoa and ova. Most species secrete and reside within, temporarily, a mucus sheath that is attached to the setae on the pleopods and hairs of endopodites of ovigerous decapods. Embryos hatch as hoplonemertean planuliform larvae.

Diagnosis – Genus *Carcinonemertes* Coe, 1902

The following diagnosis of the genus *Carcinonemertes* is taken from Coe [44] and modified by Santos et al. [45] and Sadeghian and Santos [27]: Members are nemerteans living as symbiotic egg predators on numerous species of Crustacea. With a reduced proboscis and a short, poorly developed rhynchocoel; armed with a central stylet and basis only; no lateral pouches or reserve stylets. No distinct muscular layers in the body wall, no distinct nerves, and without a thickened glandular epithelium. Missing cerebral sensory organs, and possess 2 ocelli. Cephalic glands massively developed. Internal and external fertilization and both occur.

Diagnosis – *Carcinonemertes conanobrieni* sp. nov.

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Body color varies from white to pale orange. The anterior end of the body can be either rounded or pointed. The posterior end can be either rounded or pointed. Worms are filiform in shape and range from 0.292 mm to 16.73 mm in length. Males are not significantly smaller than females. No accessory stylets present. Ovaries arranged in a single row on either side of the intestine. Adult worms can be found free roaming through the host's egg mass and may produce mucus sheaths that wind through the pleopod setae of gravid female hosts.

Material examined

Seventeen females, 15 males, and 4 larvae. Holotype: female taken from the egg mass of an adult Caribbean spiny lobster *Panulirus argus*. Type locality: holotype female

was taken from a gravid female lobster caught along Tennessee Lighthouse Reef off of Long Key, Florida in July 2016. Paratype specimens were taken from gravid female lobsters captured on either Critter Ridge Reef off Duck Key, Florida or Tennessee Lighthouse Reef off of Long Key, Florida in July 2016. Holotype female (USNM 1422303) and paratypes of both sexes (USNM 1422304– USNM 1422330) have been deposited with the Department of Invertebrate Zoology at the Smithsonian Institute in Washington, DC.

Etymology

This new species of *Carcinonemertes* is named after the social commentator and comedian Conan O'Brien. The physical similarities between the new species and Mr. O'Brien are remarkable; both exhibit a long and pale soma with slight tints of orange.

Description

The description of this species is based on living adults and four larvae. Measurements are given in mm as mean ± SD (range, number of specimens observed).

Female.

Body color of specimens varied from a cream to a pale orange. The gut can be either white (empty) to bright orange (full). Gonads are translucent white. Two eyes that range in color from bright orange to a 'rusty' red. Eyes are irregular in shape and may be circular, elliptical, or rhomboid; round eyes are the most common shape. Females may be

found roaming free among the egg mass of the host, encysted next to host eggs, or in mucus sheaths wound through the host's pleopods (Fig. 2.1, Fig. 2.2). Both the anterior end and the posterior end may be rounded or pointed (Fig. 2.3). Dimensions of relaxed worms 6.12 ± 4.32 mm (0.292-16.73 mm, 17) long and 0.540 ± 0.647 mm (0.246-3.02 mm, 17) wide. Single stylet on basis 0.012 ± 0.003 mm (0.008-0.019 mm, 14) long and 0.003 ± 0.001 mm (0.001-0.006 mm, 14) wide. Stylet basis 0.041 ± 0.005 mm (0.033-0.053 mm, 14) long and 0.009 ± 0.002 mm (0.006-0.012 mm, 14) wide. Stylet:basis ratio 0.296 ± 0.078 (0.158-0.429, 14) (Fig. 2.4). No accessory stylets present. Ovaries are arranged in a single row on either side of the intestinal diverticula (Fig. 2.3). All measurements for additional characters used in the species description can be found in Table 2.1.

Male.

Body color of specimens varied from a translucent white to a cream. The gut can be either white (empty) to bright orange (full). Gonads are translucent white. Two eyes that range in color from bright orange to a 'rusty' red. Eyes are irregular in shape and may be circular, elliptical, or rhomboid; round eyes are the most common shape. Males may be found roaming free among the egg mass of the host, encysted next to host eggs, or in mucus sheaths wound through the host's pleopods (Fig. 2.1, Fig. 2.2). Both the anterior end and the posterior end may be rounded or pointed (Fig. 2.3). Dimensions of relaxed worms 7.03 ± 3.41 mm (2.35-12.71 mm, 15) long and 0.253 ± 0.0420 mm (0.157-0.331 mm, 15) wide. Single stylet on basis 0.010 ± 0.003 mm (0.006-0.016 mm, 15) long and

0.003 ± 0.001 mm (0.001-0.006 mm, 15) wide. Stylet basis 0.043 ± 0.003 mm (0.039-0.048 mm, 15) long and 0.009 ± 0.002 mm (0.006-0.013 mm, 15) wide (Fig. 2.4). Stylet:basis ratio 0.241 ± 0.076 (0.139-0.407, 15). No accessory stylets present. Seminal vesicle 0.408 ± 0.188 mm (0.25-0.9 mm, 12) long. All measurements for additional characters used in the species description can be found in Table 2.1.

Larva.

The bodies of larvae are ciliated with both an anterior and posterior ciliary tufts (Fig. 2.4). The body shape can be either ovoid (extended) or spherical (contracted). Larvae possess two eyes, orange in color, which may be either circular or elliptical. Dimensions of the larval body are 0.115 ± 0.005 mm (0.113-0.123 mm, n=4) long and 0.051 ± 0.018 mm (0.043-0.078 mm, n=4) wide.

Quantitative and body part measurements.

Male *C. conanaobrieni* had a mean body size of 7.03 ± 3.41 mm and ranged in length between 2.35-12.71 mm; female *C. conanobrieni* had a mean body size of 6.12 ± 4.32 mm and ranged in length from 0.292-16.73 mm (Fig. 2.5). A t-test showed that there was no significant difference between the mean body size of male and female worms (t=0.6550, d.f.=30, P=0.2587).

I explored the relationship between stylet characteristics (stylet length, basis length, and stylet:basis ratio) and maximum body length for male and female *C. conanobrieni* and found no significant correlation between the variables in all instances

[stylet length (b=-0.00008, R=0.0102, t=-9.289, df=1,14, P<0.00001; b=-0.0001, R=0.0412, t=-16.947, df=1,13, P<0.00001, for males and females respectively), basis length (b=0.0001, R=0.0161, t=-32.1232, df=1,14, P<0.00001; b=-0.0005, R=0.2340, t=-39.347, df=1,13, P<0.00001, for males and females respectively), and stylet:basis ratio (b=-0.00022, R=0.0101, t=-7.912, df=1,14, P<0.00001; b=-0.0002, R=0.0001, t=-14.795, df=1,13, P<0.00001, for males and females respectively)] (Fig. 2.6). Overall, the size of the stylet remained the same irrespective of worm body size.

Furthermore, when looking to see if sexual dimorphism played a role in the size of these stylet characters, I found no evidence of such. An ANCOVA looking at the relationship between sex, body length, and stylet length showed there was no effect of sex (F=2.4368, df = 1, 28, P=0.1311) or body length (F=0.711, df = 1, 28, P=0.4071) and the interaction term was not significant (F=0.1085, df = 1, 28, P=0.7446). This indicates that neither body size nor sex has an impact on the length of the stylet. An ANCOVA looking at the relationship between worm body size, sex, and basis length showed there was no effect of sex (F=1.8877, df = 1, 28, P=0.1817) or of body size (F=0.3416, df=1, 28, P=0.5642) and the interaction term was not significant (F=1.1812, df = 1, 28, P=0.2875). The ANCOVA looking at the interaction between sex, body size, and stylet:basis ratio showed that there was no effect of sex (F=3.1379, df = 1, 28, P=0.0887), there was no effect of body size (F=0.2949, df = 1, 28, P=0.5919), and that the interaction term was not significant (F=0.3873, df = 1, 28, P=0.5393). Meaning, that regardless of body size or sex, worms exhibit the same stylet:basis relationship.

Phylogenetic Analysis.

Both maximum likelihood and Bayesian inference analyses clustered my two samples of *C. conanobrieni* together (100 and 1.0 bootstrap and support values from ML and BI analyses, respectively) and separated them from all other available COI sequences for other *Carcinonemertes* spp, *Ovicides* sp, and the selected outgroups. This indicates that *C. conanobrieni* is in fact a genetically distinct entity from all other species for which there are COI sequences available (Fig. 2.7). The genetic distance (p-value) between the two *Carcinonemertes conanobrieni* specimens was only 0.003 while the distance between *Carcinonemertes conanobrieni* specimens and representatives from other species in the phylogenetic analysis was much greater, ranging from 0.038 to 0.158.

Behavior.

Mature specimens were found either free-roaming or ensheathed within the egg mass of host lobsters (Fig. 2.1). Immature specimens were found either free-roaming or encapsulated next to a single lobster embryo (Fig. 2.4). When removed from the egg mass and placed in a Petri dish filled with seawater, nemertean specimens would either attach themselves to the glass interior or swim along the top of the water. Worms could produce copious amounts of mucus while in the Petri dish and were sometimes found grouped together on floating ‘sheets’ of mucus at the water’s surface. Specimens could move relatively quickly around the edges of the petri dish, though when mechanically disturbed, they could be slow to respond. The most common response to mechanical disturbance was to move the body either forward or backwards to evade the forcep’s tip.

Sometimes, a specimen would wrap itself around the forceps tip and adhere to it with mucus. When placed into the $MgCl_2$ solution worms would quickly coil into a spiral and produce enough mucus to coat the entire body (this layer had to be gently stripped away with forceps prior to measurements being taken). The worm specimens were fragile and great care had to be taken not to tear them when moving or adjusting them with forceps.

Ecology.

This worm is symbiotic with the Caribbean spiny lobster, *P. argus*, and may even be considered an obligatory parasite or micropredator since all life stages were observed within the brood masses of its hosts, and because worms have been shown to diminish reproductive output in infected lobsters [32] (Fig. 2.1, Fig. 2.2). Mature female worms lay mucus encased eggs throughout the lobster broods; these egg cases have a smooth surface and can be either spherical in shape or long strings entwined through the lobster's setae (Fig. 2.2). These worms also produce a mucus sheath that covers the body of the worm and is wound throughout the lobster's setae (Fig. 2.1). This sheath is usually the same length of the worm or slightly longer, it is also decorated across its surface with protruding 'hooks.'

Host and parasite distribution.

Carcinonemertes conanobrieni were found on gravid *P. argus* females from all sites in the Florida Keys that the lobsters were sampled. Worms were almost exclusively found within the brood masses of their hosts, and observed only once on the abdomen of

a female that had hatched her embryos between the time of collection and parasite examination. Though the presence of the worm was exclusive to the abdominal brooding space and its content, other body parts of lobsters of different sex and ontogenetic stages cannot be ruled out as potential microhabitats also capable of harboring worms at this time.

Taxonomic remarks.

The species described above aligns with the diagnosis of both Carcinonemertidae [27, 43] and *Carcinonemertes* [27, 44, 45] as being gonochoric with the absence of accessory stylets and pouches. In the following, I discuss the differences between *C. conanobrieni* and all previously described species within the genus *Carcinonemertes*. *Carcinonemertes conanobrieni* exhibits distinct differences from *Carcinonemertes* species that may be considered sympatric (Table 2.2), *Carcinonemertes* species that have been found to infect other lobster species (Table 2.3), as well as all other described *Carcinonemertes* species (S2.1 Table).

Carcinonemertes carcinophila carcinophila, a sympatric species, differs from *C. conanobrieni* in terms of maximum body length, ocelli characteristics, mucus sheath ornamentation, shape of egg cases, host specificity, and infestation site (Table 2.2). *Carcinonemertes c. carcinophila* has a reported maximum body length of 70 mm [44] while the maximum for *C. conanobrieni* is 16.73 mm. The two ocelli of *C.c. carcinophila* are described as being elliptical in shape and black in color [27], while those of *C. conanobrieni* vary both in shape (irregular, circular, elliptical) and color (bright orange to rusty red). *Carcinonemertes carcinophila carcinophila* produces mucus

sheaths that display lapilli cells across the sheath [45]. In contrast, *C. conanobrieni* has hooks protruding along the sheath. Eggs laid by *C.c. carcinophila* are distributed throughout the brood mass of host crabs in long strings [28]. *Carcinonemertes conanobrieni* instead lays eggs in the brood mass of the host both in long strings and in nearly perfectly spherical sacs. Furthermore, *C.c. carcinophila* does not appear to be host specific, with it having been found infecting at least 28 different crustacean hosts [27, 28]. *Carcinonemertes carcinophila carcinophila* also infects both male and female crab hosts and may be found within the gill chambers or on the brood masses of female crabs [46]. Thus far, *C. conanobrieni* has only been found on gravid (or recently gravid) female *P. argus*, and only within the brooding space, although additional studies are needed to confirm these preliminary observations.

A second sympatric species, *Carcinonemertes carcinophila imminuta*, differs from *C. conanobrieni* in terms of body length, body width, ocelli characteristics, mucus sheath ornamentation, number of ovaries, shape of egg cases, host specificity, and infestation site (Table 2.2). The maximum reported body length for *C. c. immunuta* is 35 mm for females and 16 mm for males [27] while the maximum body length of *C. conanobrieni* is 16.73 mm for females and 12.71 for males. Maximum body width of *C.c. imminuta* females is 0.22 mm and males is 0.214 mm [27]; *C. conanobrieni* has a maximum body width of 3.02 mm for females and 0.331 mm for males. Furthermore, while adult *C.c. imminuta* have 2 irregular shaped eyes colored with yellowish-brown, brown, or black and larvae have 4 irregular shaped eyes of the same color [27], *C. conanobrieni* has two irregular shaped eyes both as a larva and as an adult. *Carcinonemertes carcinophila imminuta* and *C. conanobrieni* both produce ornamented mucus sheaths, though *C.c. imminuta* displays lapilli cells [45] while *C. conanobrieni* has hooks arranged along the sheath. The largest measured female *C.c. imminuta* has a reported 370 ovaries [27] while *C.*

conanobrieni averages 87.4 ± 43.6 ovaries. Eggs laid by *C.c. imminuta* are positioned throughout the brood mass of host crabs in long strings. *Carcinonemertes conanobrieni* also lays eggs in the brood mass of the host in long strings, but also in nearly perfect spherical sacs. *Carcinonemertes carcinophila imminuta* does not exhibit host specificity and has been found on multiple crustacean hosts [27] and is reported on both male and females, as well as on immature and mature crabs. As stated above *C. conanobrieni* so far has only been found on gravid or recently gravid female *P. argus*.

Carcinonemertes pinnotheridophila, a third sympatric species, differs from *C. conanobrieni* in terms of body length, ocelli characteristics, mucus sheath, basis length, stylet:basis ratio, and in host of choice (Table 2.2). *C. pinnotheridophila* has a smaller maximum body size reported at 8.4 mm for females and 2.3 mm for males [47], while *C. conanobrieni* has a maximum body size of 16.73 mm in females and 12.71 mm in males. *Carcinonemertes pinnotheridophila* lack ocelli at any life stage, while *C. conanobrieni* have two ocelli throughout their lives. Furthermore, while *C. pinnotheridophila* does secrete a mucus sheath, this sheath is not ornamented, *C. conanobrieni* secretes a mucus sheath ornamented with hooks. The length of the basis is 0.016 mm for female *C. pinnotheridophila* and 0.0181 mm for males, which is considerably smaller than what I have found for *C. conanobrieni* with a mean basis length of 0.041 ± 0.005 mm for females and 0.043 ± 0.003 for male worms. The stylet:basis ratio of *C. pinnotheridophila* was 0.5 for females and 0.365 for males, while in *C. conanobrieni*, the ratio was 0.296 ± 0.078 mm for females and 0.241 ± 0.087 for males, which is smaller. While both *C. pinnotheridophila* and *C. conanobrieni* are seemingly host-specific, they differ in their chosen hosts. *Carcinonemertes pinnotheridophila* are found in the egg masses of brooding *Pinnixa chaetopterana* as well as in the branchial chamber of non-brooding females.

Though *C. pinnotheridophila* is only reported to infect *Pinnixia chaetopterana*, it shares characteristics that are similar to the undescribed *Carcinonemertes* spp. that infect *Zaops ostreum* (in North Carolina) and *Austinixa gorei* (in Florida) [47]. *Carcinonemertes conanobrieni* has thus far only been found in association with brooding female *P. argus*.

The newly described species also exhibits characteristics that distinguish it from the two other species of *Carcinonemertes* that have been found to infect lobsters (Table 2.3). One such species, *Carcinonemertes wickhami*, differs from *C. conanobrieni* in terms of body size and sexual dimorphism, ocelli characteristics, distance between ocelli, distance from ocelli to the tip of the head, and mucus sheath production (Table 2.3). *Carcinonemertes wickhami* displays noticeable sexual dimorphism with females having a range of body lengths from 10-30 mm while males range from 5-18 mm [23]. On the other hand, *C. conanobrieni* displays little sexual dimorphism with females exhibiting a range in size from 0.292-16.73 mm and males from 2.35-12.71 mm. *C. wickhami* have two eyes that are black in color and cup shaped [23] while those of *C. conanobrieni* vary both in shape (irregular, circular, elliptical) and color (bright orange to rusty red). Female *C. wickhami* have eyes that are 0.257 mm apart and 0.145 mm to the tip of the head, males have eyes that are 0.162 mm apart and 0.163 mm from the tip of the head. The mean distance between the eyes for *C. conanobrieni* is 0.087 ± 0.025 mm for females and 0.077 ± 0.022 mm for males which is more narrow than *C. wickhami*; the distance from the eyes to the tip of the head was 0.166 ± 0.041 mm for females and 0.175 ± 0.031 mm for males which is larger than for *C. wickhami*. C*arcinonemertes wickhami* also produces lapilli-covered mucus sheaths which differ from the hook-ornamented sheaths of *C. conanobrieni*. As I have reported for *C. conanobrieni*, *C. wickhami* worms may also be found on the egg-bearing pleopods of

female lobsters and additionally at the base of the uropods; with no reports from male lobsters and juveniles lobsters [16, 23].

Carcinonemertes australiensis infects the spiny lobster *Panulirus cygnus*, and differs from *C. conanobrieni* in both body width and mucus sheath production (Table 2.3). The reported body dimensions of a single *C. australiensis* individual are 7 mm long and 1 mm wide [24]. *Carcinonemertes conanobrieni* displays a mean width of 0.540 ± 0.647 mm in females and 0.235 ± 0.0420 mm in males, both of which are smaller than what is reported for *C. australiensis*. In contrast to *C. conanobrieni*, there is no report of a mucus sheath being produced by *C. australiensis*. In agreement with other lobster-infecting species, *C. australiensis* has been reported to inhabit only the egg masses of brooding females [16, 24].

S2.1 Table shows the differences between *C. conanobrieni* and all remaining described species of *Carcinonemertes*. With a mean body length and range of 7.03 ± 3.41 mm (2.35-12.71 mm) for male worms and a mean body length and range of 6.12 ± 4.32 mm (0.292-16.73 mm) for female worms, *C. conanobrieni* is considerably smaller than *Carcinonemertes mitsurii* (max 100 mm [males] and max 300 mm [females]) [27]. *Carcinonemertes conanobrieni* is reported as being larger than *Carcinonemertes divae* (2.6 ± 0.2 mm (males) and 2.6 ± 0.1 mm (females)) [45], *Carcinonemertes caissarum* (2.0 ± 0.3 mm (males) and 5.5 ± 1.0 mm (females)) [45], *Carcinonemertes regicides* (1.6 mm (males) and 2.1 mm (females)) [23, 29], *Carcinonemertes kurisi* (1.8 ± 0.1 mm (males) and 4.5 ± 0.3 mm (females)) [29], and *Carcinonemertes tasmanica* (1.9 ± 0.7 mm (males) and 5.6 ± 1.3 mm (females)) [29]. Furthermore, *C. conanobrieni* does not exhibit the relatively high sexual size dimorphism (males << females) that is reported for *C. caissarum*, *C. kurisi*, *C. tasmanica*, and *C. mitsukurii* [27, 29, 45].

Carcinonemertes conanobrieni varies in body color from a translucent white to a pale orange, this aligns with many species within the genus *Carcinonemertes*, but does differ from the body colors of *C. caissarum*, where males have a red spot on the posterior end, *C. errans* (pink to reddish orange) [30, 48], *C. regicides* (pink, red-orange, and dull orange) [43], *C. epialti* (bright orange to reddish-yellow) [27, 45], *C. kurisi* females (dark orange to red-pink) [29], and *C. tasmanica* (red) [29].

With a stylet basis mean length and range of 0.043 ± 0.003 mm (0.039 - 0.048 mm) for male *C. conanobrieni* and 0.041 ± 0.005 mm (0.033 – 0.0053 mm) for female *C. conanobrieni*, the new species has a basis that is longer than almost every other species. The only exception to this is *C. regicides* which has a stylet basis length of 0.0405 mm [27, 43].

The mucus sheath with decorative hooks that is produced by *C. conanobrieni* is very different from the sheaths that are reported for other species of *Carcinonemertes*. *Carcinonemertes regicides* forms a mucus sheath that is not decorated and breaks very easily when manipulated [43], *C. kurisi* and *C. tasmanica* both produce distinctive corkscrew shaped sheaths [29], and *C. sebastianensis*, *C. caissarum*, and *C. diavae* produce sheaths covered in lapilli [45].

Finally, *C. conanobrieni* differs from all species mentioned in S2.1 Table in that they have been found infecting the Caribbean spiny lobster, *P. argus*. Thus far, *C. conanobrieni* is the only species of *Carcinonemertes* reported to infect *P. argus*. The species of *Carcinonemertes* found in S2.1 Table have all been found in crab hosts, and host specificity differs in these species. *C. errans* has been shown to be extremely host-specific, while *C. mitsukurii* [27], C. *divae* [45], *C. caissarum* [45], *C. sebastianensis* [45], *C. kurisi* [45, 29], and *C. tasmanica* [29]

have all been reported on a single host. *C. epialti* shows host preference, but is not specific to a single crab species.

As previously stated, the considerable amount of morphological homogeneity in the genus *Carcinonemertes* has in the past made species identification both difficult and at times unclear [24, 27, 45]. This difficulty arises from small size of the worms, the similarities in ecology and morphology driven by a parasitic lifestyle, and the ambiguity that comes from distinguishing closely linked morphological structures [45]. In addition, the use of these ambiguous structures for initial identification can make future identification more difficult. I feel that some structures that have been used in the past for species differentiation are not well suited for the task. For instance, using the shape of the posterior and anterior ends of the worms (as in [45]) may lead to confusion in some cases. I found that the shape of the ends of *C. conanobrieni* showed some variation as a result of how relaxed the specimen was, if the worm was fully extended or not, and the amount of water present under the cover-slip. The ocelli, a character often used in species descriptions [24, 27, 48] is another example of a character with too much ambiguity. I found that there was variation in the color of the eyes of *C. conanobrieni*, more light led to eyes that were bright orange in color, and less light to a rusty-red. This difference in eye coloration of many of *Carcinonemertes* species can most likely be attributed to different light intensities. This should be taken into consideration when using eye color as a diagnostic character. Furthermore, the shape of the eyes varied with the orientation of the worm and with whether or not a cover-slip was present. However, the presence or absence of ocelli and the number of ocelli are both clearly quantitative measures that should continue to be used.

Based on my observations and what has been to this point reported, there are a number of morphological and ecological characteristics that can offer clear distinctions between species.

Some of these include the external mucus sheath, stylet/basis characteristics, body size, number of ovaries, and host specificity. Because of the stark differences that exist for the mucus sheath, whether a sheath is produced or not, the presence or absence of lapilli cells, the presence of decorative hooks, and shape, it can be reliable means of distinguishing one species from another. Interestingly, I found that the sizes of the stylet and its basis did not change with worm body size for *C. conanobrieni*; if this is true for other species of *Carcinonemertes* then the stylet and basis can be very reliable for species differentiation. Since their sizes are not impacted by growth after sexual maturity, any significant differences that exist can clearly define a species. Body size (there is a wide reported range of sizes) and sexual size dimorphism (whether or not it occurs) can also be useful tools in separating species. Furthermore, I agree with Santos et al. [45] in that adding more measurements of practical morphological characters (i.e. number of ovaries, distance from ovaries/testes to the head, distance between the ocelli) when describing species within this group of worms will help to improve both quality description as well as the understanding of the observed extant diversity. By increasing the number of externally visible morphological characters that are measured the description and differentiation of species should become much more attainable, allowing researchers to tackle the current abundance of undescribed nemertean worms [49]. Furthermore, the addition of genetic characters will be exceedingly helpful in future studies looking to resolve the group's phylogeny (see, 49).

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Table 2.1. Additional measurements of *Carcinonemertes conanobrieni* sp. nov. used to differentiate this species from other *Carcinonemertes* species. All measurements are given in mm (exceptions include: stylet:basis ratio, a ratio with no units, and the number of ovaries is a direct count). The number in parentheses (#) following the range of measurements indicates the number of specimens measured for the data.

Character	Males			Females		
	Mean	Standard Deviation	Range	Mean	Standard Deviation	Range
Body Length	7.03	± 3.41	2.35-12.71 (15)	6.12	± 4.32	0.292-16.73 (17)
Body Width	0.253	± 0.0420	0.157-0.331 (15)	0.540	± 0.647	0.246-3.02 (17)
Eye Length	0.037	± 0.008	0.023-0.050 (15)	0.033	± 0.013	0.019-0.066 (17)
Eye Width	0.027	± 0.007	0.019-0.041 (15)	0.025	± 0.006	0.016-0.040 (17)
Distance: Between Eyes	0.077	± 0.022	0.043-0.111 (15)	0.087	± 0.025	0.054-0.143 (17)
Distance: Eyes to Tip of Head	0.175	± 0.031	0.106-0.229 (15)	0.166	± 0.041	0.083-0.211 (17)
Brain Lobe Length	0.121	± 0.019	0.08-0.155 (15)	0.128	± 0.036	0.074-0.22 (16)
Brain Lobe Width	0.084	± 0.019	0.05-0.11 (15)	0.091	± 0.034	0.053-0.195 (16)
Distance: Top of Brain to Tip of Head	0.211	± 0.043	0.13-0.29 (15)	0.218	± 0.073	0.11-0.345 (16)
Anterior Proboscis	The length continued to the top of the head and could not be accurately determined					

Chamber Length						
Anterior Proboscis Chamber Width	0.028	± 0.010	0.015-0.048 (10)	0.0246	± 0.008	0.011-0.04 (10)
Diaphragm Length	0.058	± 0.013	0.028-0.073 (15)	0.063	± 0.010	0.051-0.09 (14)
Diaphragm Width	0.050	± 0.012	0.03-0.076 (15)	0.053	± 0.0133	0.029-0.083 (14)
Proboscis Bulb Length	0.027	± 0.007	0.015-0.038 (15)	0.031	± 0.011	0.016-0.053 (14)
Proboscis Bulb Width	0.027	± 0.008	0.03-0.051 (14)	0.041	± 0.013	0.025-0.065 (14)
Posterior Proboscis Chamber Length	0.108	± 0.040	0.07-0.15 (3)	Posterior proboscis chamber measurements were only taken for three male specimens		
Posterior Proboscis Chamber Width	0.039	± 0.010	0.028-0.048 (3)			
Single Stylet Length	0.010	± 0.003	0.006-0.016 (15)	0.012	± 0.003	0.008-0.019 (14)
Single Stylet Width	0.003	± 0.001	0.001-0.006 (15)	0.003	± 0.001	0.001-0.006 (14)
Stylet Basis Length	0.043	± 0.003	0.039-0.048 (15)	0.041	± 0.005	0.033-0.053 (14)
Stylet Basis Width	0.009	± 0.002	0.006-0.013 (15)	0.009	± 0.002	0.006-0.012 (14)
Stylet:Basis Ratio	0.241	± 0.076	0.139-0.407 (15)	0.296	± 0.078	0.158-0.429 (14)

Distance: Tip of Stylet to Tip of Head	0.245	± 0.076	0.125-0.265 (14)	0.251	± 0.088	0.12-0.415 (14)
Number of Ovaries	----			87.4	± 43.6	48-186 (12)
Seminal Vesicle Length	0.408	± 0.188	0.25-0.90 (12)	----		
Distance: First Gonad to Tip of Head	0.554	± 0.169	0.3-0.85 (14)	0.691	± 0.201	0.375-1.09 (15)

Table 2.2. Comparison of morphological and ecological traits of *Carcinonemertes conanobrieni* sp. nov. to sympatric species (*Carcinonemertes carcinophila carcinophila*, *Carcinonemertes carcinophila immunta*, and *Carcinonemertes pinnotheridophila*).

Character		***C. conanobrieni***		***C. c. carcinophila***		***C. c. imminuta***		***C. pinnotheridophila***	
		Male	Female	Male	Female	Male	Female	Male	Female
Worm Body Color		Translucent White to Cream	Translucent White to Pale Orange	Yellowish-Orange, Pale Reddish, Rose Pink, Brick Red		Whitish	Reddish	Off-White or Tan	Orange-Red
Body Length		2.35-12.71 mm	0.296-16.73 mm	6.0-70.0 mm		8.68 mm (average)	16.55 mm (average)	2.3 mm (max)	8.4 mm (max)
Body Width		0.157-0.331 mm	0.246-3.02 mm	----		0.214 mm (average)	0.22 mm (average)	----	
Infestation Site		Egg Mass		Gill lamelle, Egg Mass		Gill lamelle, Egg Mass		Branchial Chamber, Egg Mass	
Ocelli Characters	Number	2		2		4 / 2		No Ocelli	
	Color	Bright Orange to Red		Black		Yellowish-Brown, Brown, Black			

	Shape	Irregular (Cup or Elliptical)		Elliptical		Irregular			
Distance from Eyes to Head		0.106-0.229 mm	0.083-0.211 mm	----		----	0.135 mm	----	
Distance between Eyes		0.043-0.111 mm	0.054-0.143 mm	----		----	0.200 mm	----	
Stylet Length		0.006-0.016 mm	0.008-0.019 mm	0.006-0.012 mm		0.006-0.0095 mm		0.0066 mm	0.008 mm
Basis Length		0.039-0.048 mm	0.033-0.053 mm	0.020-0.030 mm		0.019-0.023 mm		0.0181 mm	0.016 mm
Stylet:Basis Ratio		0.139 -0.407	0.158-0.429	0.316-0.400		0.0461		0.365	0.5
Mucus Sheath		Yes (ornamented)		Yes (ornamented)		Yes		Yes	
Egg Sheath Shape		----	Long Strands or Spherical Cases	----	Long Strands	----	Long Strands	----	Spherical Cases

Table 2.3: Comparison of morphological and ecological traits of *Carcinonemertes conanobrieni* sp. nov. to *Carcinonemertes* species that have been found on other species of spiny lobster (*Carcinonemertes wickhami* and *Carcinonemertes australiensis*).

Character		***C. conanobrieni***		***C. wickhami***		***C. australiensis***	
		Male	Female	Male	Female	Male	Female
Worm Color		Translucent White to Cream	Translucent White to Pale Orange	Pinkish-White	Orange	Translucent White	
Body Length		2.35-12.71 mm	0.296-16.73 mm	5-18 mm	10-30 mm	7 mm	
Body Width		0.157-0.331 mm	0.246-3.02 mm	0.400 mm		1 mm	
Infestation Site		Egg Mass		Egg Mass		Egg Mass	
Ocelli Characters	Number	2		2		2	
	Color	Bright Orange to Red		Black		Black	
	Shape	Irregular (Cup or Elliptical)		Cup		----	
Distance from Eyes to Head		0.106-0.229 mm	0.083-0.211 mm	0.163 mm	0.145 mm	----	
Distance between Eyes		0.043-0.111 mm	0.054-0.143 mm	0.162 mm	0.257 mm	----	
Stylet Length		0.006-0.016 mm	0.008-0.019 mm	0.019-0.200 mm		0.015-0.018 mm	

Basis Length	0.039-0.048 mm	0.033-0.053 mm	0.036-0.042 mm		0.040 mm	
Stylet:Basis Ratio	0.139 - 0.407	0.158-0.429	0.476-0.528		0.375-0.45	
Mucus Sheath	Yes (ornamented)		Yes (ornamented)		No	
Egg Sheath Shape	----	Long Strands or Spherical Cases	----	Long Strands	----	Not Reported

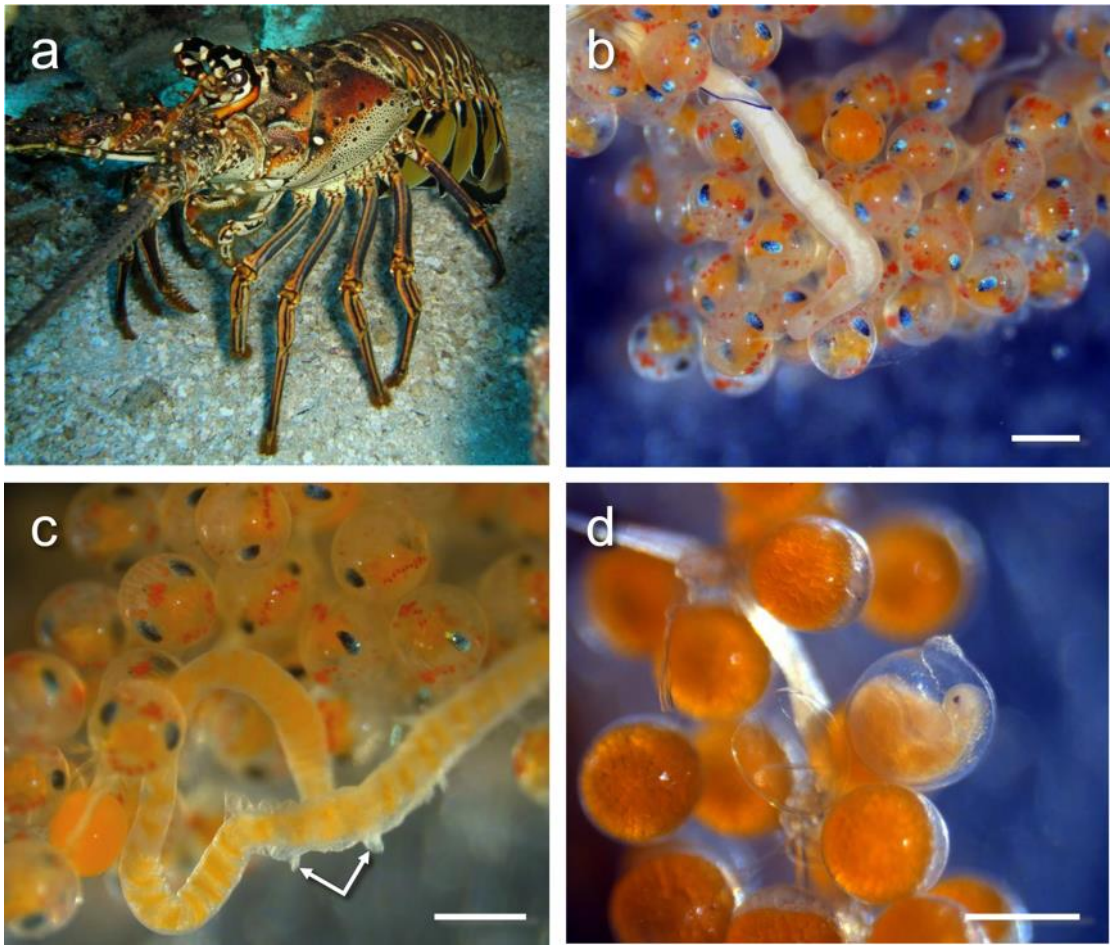

Fig. 2.1. *Panulirus argus* and representative photographs of *Carcinonemertes conanobrieni*. (a) Shows a female *P. argus* on a reef in the Florida Keys, the remaining photographs are representative of some of the different ways *Carcinonemertes conanobrieni* may be found within the lobster brood mass [the scale bars in photos b, c, and d all indicate 0.5 mm]. (b) Male *C. conanobrieni* free-roaming among late stage lobster embryos. (c) Female *C. conanobrieni* partially covered by a mucus sheath with decorative hooks (indicated by arrows) protruding. (d) *C. conanobrieni* of undetermined sex encapsulated next to early stage lobster embryos.

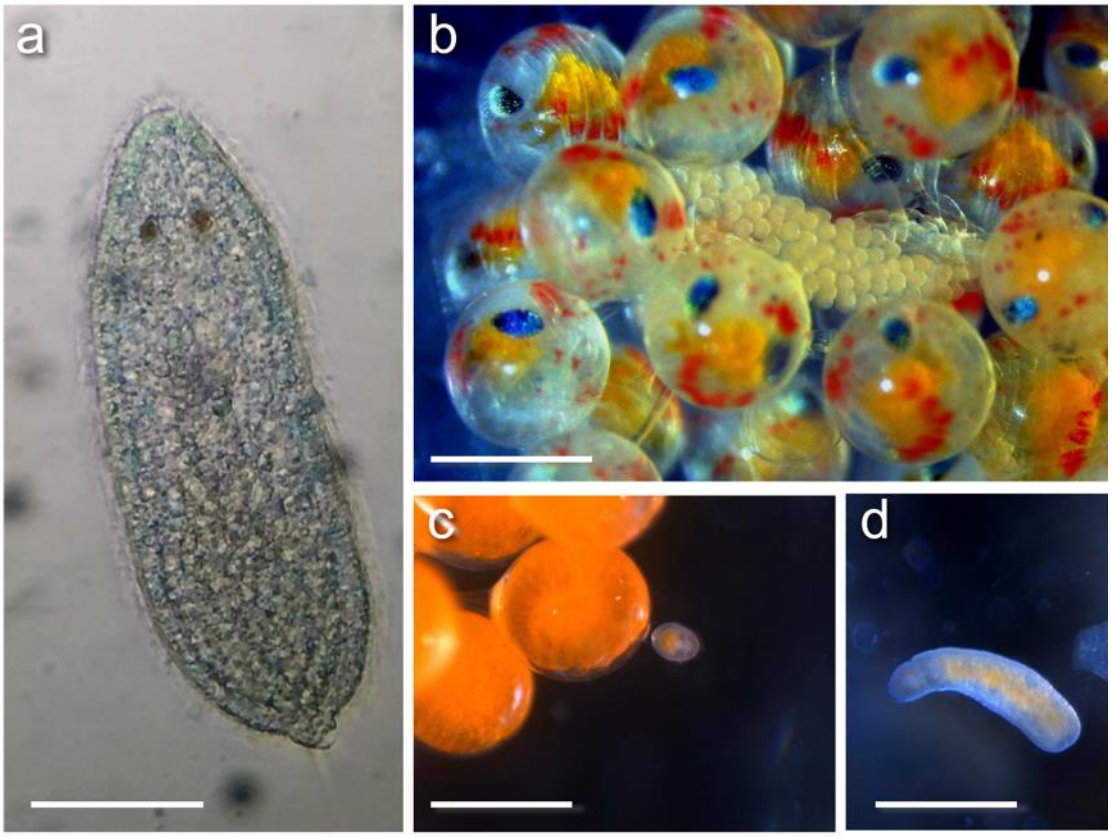

Fig. 2.2. *Carcinonemertes conanobrieni* hoplonemertean larvae, egg cases, and early juveniles. (a) Shows a dorsal view of a hoplonemertean larvae that had been stained with methylene blue for better contrast [scale bar represents 0.05 mm]. (b) A string of *C. conanobrieni* embryos wound through late stage lobster embryos [scale bars in b, c, d represent 0.5 mm]. (c) A juvenile *C. conanobrieni* encysted next to an early stage lobster embryo. (d) A newly emerged juvenile *C. conanobrieni* worm from its cyst attached to a lobster embryo.

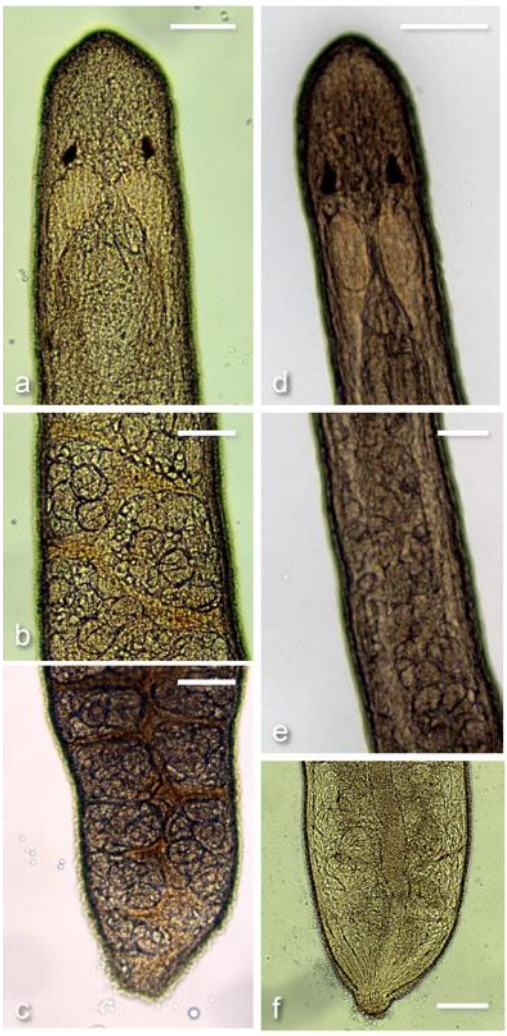

Fig. 2.3. Representative body segments of male and female *Carcinonemertes conanobrieni*. Each vertical set of photos shows sections of the anterior, trunk, and posterior for a female (left) and male (right) *C. conanobrieni* [the scale bar in each photograph represents 0.1 mm]. (a) And (d) show the anterior portions of a female and male worm with ocelli, cerebral lobes, and stylet all visible. (b) Shows a section of the trunk of a female *C. conanobrieni* with full ovaries separated by the intestinal diverticula, and (e) depict a section of a male's trunk with testes distributed throughout. (c) Is the posterior end of a female, which has ovaries present for the entire length and (f) is the posterior end of a male with testes stopping just prior to the seminal vesicle (not clearly visible).

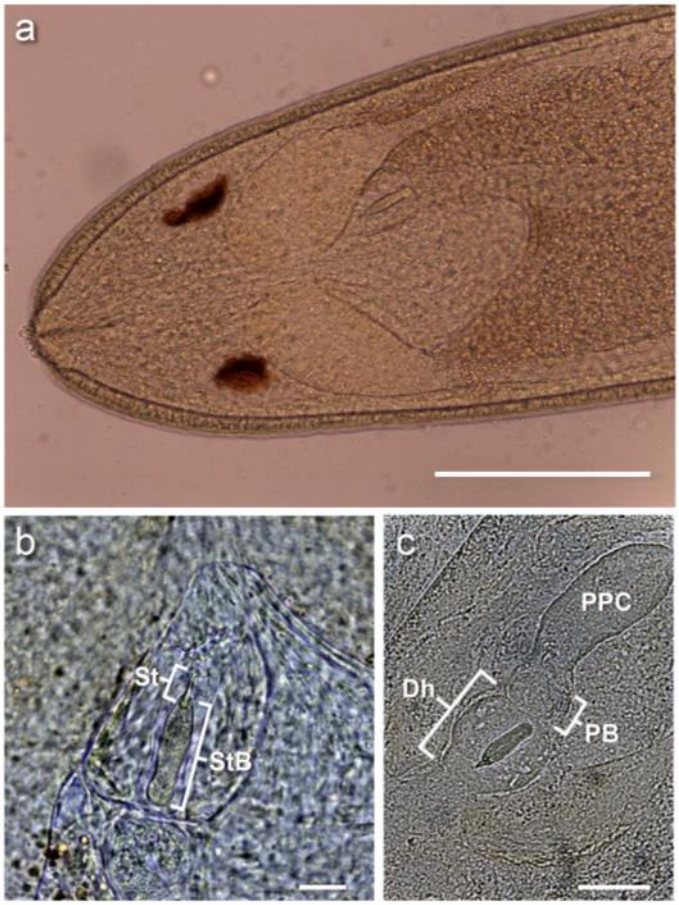

Fig. 2.4. Anterior end of *Carcinonemertes conanobrieni* with a focus on the stylet and surrounding regions.

(a) Ventral view of the anterior section of a male *C. conanobrieni*; the stylet is positioned just below the right cerebral lobe and is slightly angled [the scale bar represent 0.2 mm]. (b) A slightly angled stylet [St] and stylet basis [StB] [scale bar represent 0.02 mm]. (c) A clear depiction of the stylet, stylet basis, posterior proboscis chamber [PPC], proboscis bulb [PB], diaphragm [Dh], and part of the anterior proboscis chamber.

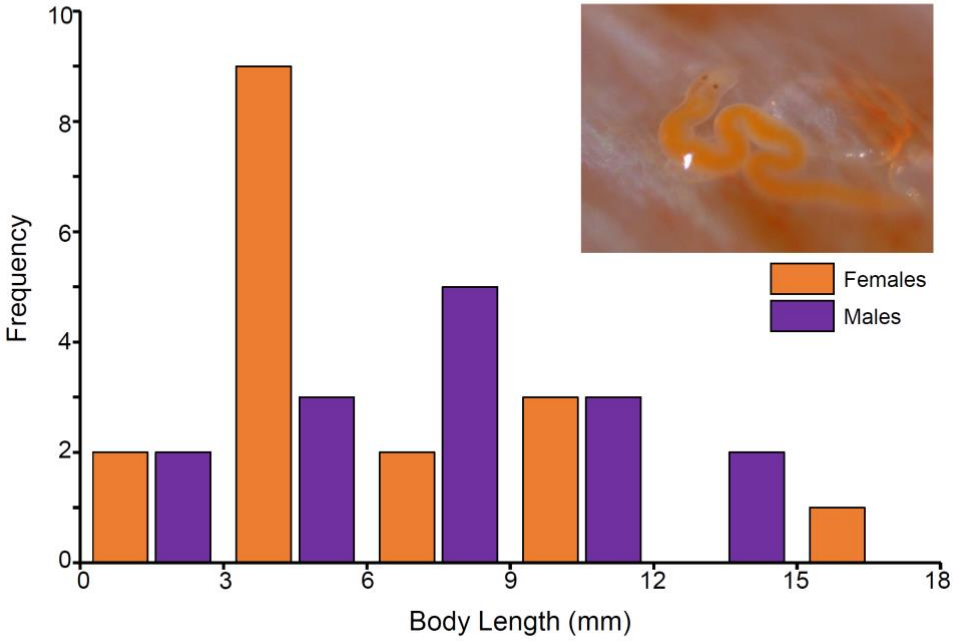

Fig. 2.5. Size-frequency distribution of male and female *Carcinonemertes conanobrieni*. Male body size ranged from 2.35 to 12.71 mm (mean, 7.03 ± 3.41 mm) and female body size ranged from 0.292 to 16.73 mm (mean, 6.12 ± 4.32 mm). In the upper-right is a female *C. conanobrieni* within the brood mass of its lobster host.

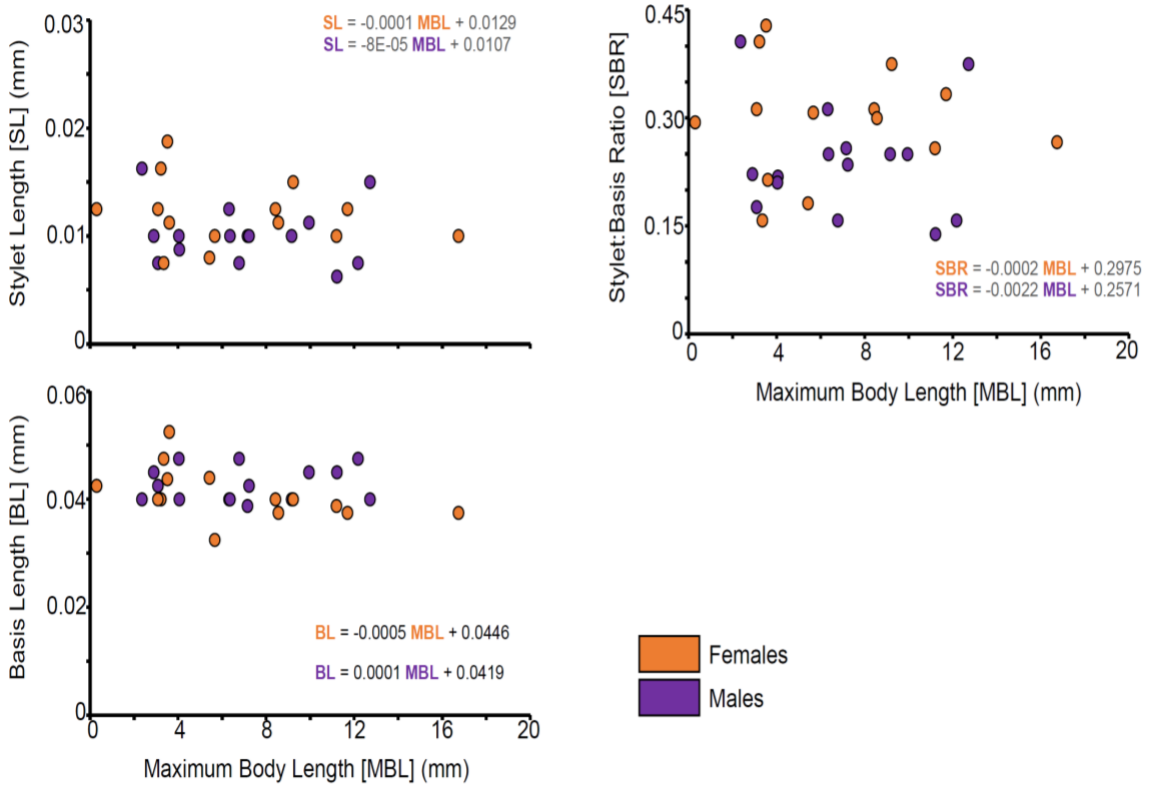

Fig. 2.6. Relationship between stylet characteristics and maximum body length [MBL] for male and female worms.

(a) relationship between MBL and stylet length [SL] for both sexes. (b) relationship between MBL and the stylet:basis ratio [SBR] for both sexes. (c) the relationship between MBL and basis length [BL] for both sexes. In all instances there was no impact of sex on these relationships.

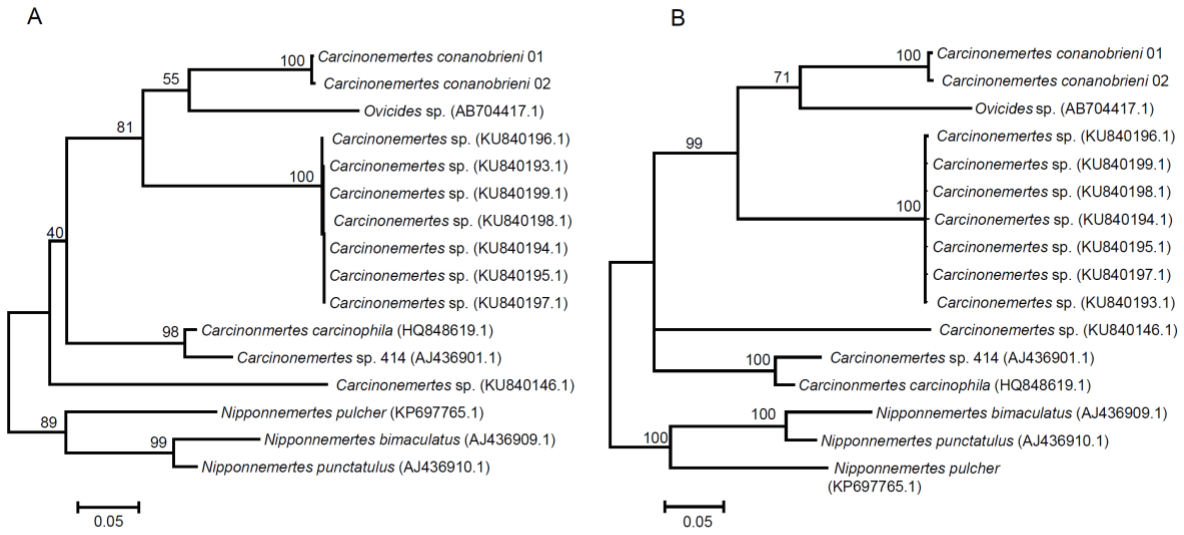

Fig. 2.7. Maximum likelihood and Bayesian inference phylogenetic trees.

A maximum likelihood tree (a) and a Bayesian inference tree (b) both depict the phylogenetic relationship between the sp. nov. (*C. conanobrieni*) and all *Carcinonemertes* species where COI sequences were available. Outgroup species used include *Ovicides* sp., *Nipponnemertes punctatus*, *Nipponnemertes bimactulata*, and *Nipponnemertes pulchra*. Both trees show clear separation between the spe. nov. and all other species used in the anaylses. Accession numbers for GenBank are listed in parenthesis next to the species names.

S2.1 Table: Comparison of morphological and ecological traits of *Carcinonemertes conanobrieni* sp. nov. to *Carcinonemertes* species that are considered non-sympatric and are found on non-lobster hosts. Morphological measurements from *Carcinonemertes mitsukurii*, *Carcinonemertes divae*, *Carcinonemertes caissarum*, *Carcinonemertes sebastianensis*, *Carcinonemertes coei*, *Carcinonemertes errans*, *Carcinonemertes regicides*, *Carcinonemertes humesi*, *Carcinonemertes epialti*, *Carcinonemertes kurisi*, and *Carcinonemertes tasmanica* taken from the literature for a comparative table. Supplementary information may be found in association with the following link: https://doi.org/10.1371/journal.pone.0177021.

S2.2 Table: Raw morphological measurements for *Carcinonemertes conanobrieni*, sp. nov. Supplementary information may be found in association with the following link: https://doi.org/10.1371/journal.pone.0177021.

S2.3 Text. COI sequences of two *Carcinonemertes conanobrieni* sp. nov. specimens used for phylogenetic analysis. Supplementary information may be found in association with the following link: https://doi.org/10.1371/journal.pone.0177021.

Chapter 3

Host-Use of *Panulirus argus* by *Carcinonmertes conanobrieni* and Implications of Infection on the Reproductive Performance of the Host

Abstract.

Panulirus argus, the Caribbean spiny lobster, plays host to a number of different infections and parasites, including the newly described nemertean, *Carcinonemertes conanobrieni*. To determine the host use, infection prevalence, and infection intensity of this new parasite on *P. argus*, male, non-gravid female, and gravid female lobsters were captured along the Florida Key reef tract from and examined for *C. conanobrieni* infection. Furthermore, infected gravid females were also used in estimating the impact that infection by this nemertean had on three levels of reproductive performance (reproductive output, fecundity, and brood mortality). We found that all male lobsters (n=30) and all but two non-brooding female lobsters (n=30) showed no signs of infection by this nemertean worm, while all but 7 out of 114 sampled gravid female lobsters were infected by *Carcinonemertes conanobrieni*. When investigating the impact that infection had on the reproductive performance of gravid females, we found that the interaction between intensity of infection and embryo stage had a significant impact on female fecundity ($F=7.1792$, d.f.= 1, 74, $P= 0.0092$). The interaction between egg stage and infection status was marginally significant on reproductive output ($F=3.68$, d.f.= 1, 74, $P= 0.0591$). We also found that there was no effect of infection on brood mortality of female lobsters. We believe that C. conanobrieni has the potential to have a significant impact on the health of the lobster fishery in the Florida Keys, and the presence this worm should be taken into account when considering new fishery management strategies.

Introduction.

Marine systems are one of the most valuable natural environments worldwide providing important commercial and ecosystem services (e.g. CO_2 absorption, water filtration, shoreline protection, nursery and feeding grounds to commercially important fish, and tourism services) (Suttle, 2007; Hoegh-Guldberg & Bruno, 2010; Staudinger et al., 2013; Ruckelshaus et al., 2013). However, these systems are vulnerable and easily influenced by both natural and human actions (Gilman et al., 2008). Recent and severe mass mortalities of fishes, corals, sponges, and other invertebrates in marine environments have led to an increase in research focusing on the health of the oceans, and changes in disease outbreaks in particular (Harvell et al., 1999; Hayes et al., 2001; Lafferty et al., 2004; Ward & Lafferty, 2004). Evidence does show that there may be a trending global increase in disease in marine environments over the last decades (Ward & Lafferty, 2004; Lafferty, 2004). Even with this global increase there appear to be some areas that can be considered disease hotspots, where new diseases are emerging at an even higher prevalence than other areas (Harvell et al., 2007). The wider Caribbean region is considered one such area, and over the past 25 years it has seen a rapid increase coral bleaching events, new and virulent disease emergence, and in infectious disease outbreaks (Harvell et al., 2007; Weil et al., 2009; Doney et al., 2012; Ruiz-Moreno et al., 2012).

Though most forms of disease have seen a rise in occurrence in the wider Caribbean region over the last few decades, one area of interest is parasitism (Ward & Lafferty, 2004; Shields, 2011). Infection by disease or infectious organisms (parasites) has been shown to have a variety of negative effects on the health of a host. Growth, longevity, reproduction, egg survival, and marketability all may be impacted for a host once it is infected by a parasite (Kuris et al., 1991). Parasitic agents include microbial diseases (i.e. bacteria, fungi, protozoans, and viruses)

that build infectious population sizes very quickly (Kuris et al., 1991) and castrators (i.e. rhizocephalan barnacles or epicaridean isopods) (Kuris, 1974) that may chemically halt the reproduction of its host (Kuris, 1991; Ebert et al., 2004). Parasites may also feed on the embryos of their hosts (i.e. nemertean worms) that at high levels of infection may lead to reproductive failure in host populations (Wickham, 1986; Shields & Kuris, 1988; Kuris et al., 1991 a,b). Crustaceans in particular are often targeted by parasites, and there are groups of parasites that have specialized in crustacean hosts. One such group is comprised of worm species belonging to the family Carcinonemertidae within the phylum Nemertea (Giribet, 2008).

The family Carcinonemertidae contains the genera *Ovicides* and *Carcinonemertes* (Humes, 1942; Shields et al., 1989). There are currently 5 described species of *Ovicides* and 17 described species of *Carcinonemertes* found in association with approximately 70-76 recorded host species (Humes, 1942; Wickham & Kuris, 1985; Shields & Segonzac, 2007), with most occurring on cancrid, portunid, and xanthid crabs as well as on panulirid lobsters (Campbell et al., 1989; Shields & Segonzac, 2007; Sadeghian & Santos, 2010). Host specificity varies within the family, and some species will infect only a single host such as *C. errans* on *Cancer magister* (Wickham, 1996) and *O. juliaea* on *Chlorodiella nigra* (may be found in rare occasions on *C. xishanensis*) (Shields, 2001); while others have been reported on more than a dozen decapod species of crab (*C.c. carcinophila*, *C.c. imminuta*, and *C. epialti*) (Humes, 1942; Shields & Segonzac, 2007). All Carcinonemertidae worms are considered voracious egg-predators and epidemic levels of infection by species within the genus have led to reproductive failure in host populations as well as to the collapse of a few fisheries (Wickham, 1980; Wickham and Kuris, 1985).

Though there is a relatively high number species within the family, research into the impact of these worms on their hosts has been limited to a few species, usually in temperate regions (one exception is *Carcinonemertes mitsukurii* infecting *Portunus pelagicus*), and usually to those that directly affect commercially important hosts (Wickham, 1979; Shields & Kuris, 1988; Shields et al., 1990). The Dungeness crab, *Cancer magister*, is one host that has experienced a significant impact of infection by *Carcinonemertes errans*. It has been demonstrated that a single *C. errans* worm can consume an average of 70 embryos over one single brooding period, and that infection by this worm has resulted directly in the mortality of 55% of *C. magister* embryos (Wickham, 1979). Another well studied *Carcinonemertes* parasite, *C. epalti*, has been found on both the yellow rock crab, *Cancer anthonyi* (Shields et al., 1990) and the shore crab, *Hemigrapsus oregonensis* (Shields & Kuris, 1988). The impact of infection on these two hosts varied little during non-outbreak sampling, with *C. anthonyi* experiencing a mean egg loss of 5.7% during the sampled period (Shields et al., 1990) and *H. oregonensis* experiencing a mean egg loss of 5.6% in non-outbreak years (Shields and Kuris, 1988). However, during an infection outbreak (i.e. high infection prevalence and intensity) *H. oregonensis* experienced 75-100% egg loss (Shields and Kuris, 1988). Kuris et al. (1991) looked at the impact of infection by *Carcinonemertes regicides* on brood mortality of the red king crab, *Paralithodes camtschatica*. They found that the abbreviated life cycle and autoinfection of *C. regicides* along with the progression of the breeding season could lead to a greater than 90% brood loss and a possible reduction or elimination of recruitment of some year classes within the crab fishery (Kuris et al., 1991). Lastly, *Carcinonemertes mitsukurii* which was found infecting *Portunus pelagicus* had no measurable impact on egg mortality (Shields & Wood, 1993).

Recently, it has been found that a new species of *Carcinonemertes*, *Carcinonemertes conanobrieni*, is infecting the Caribbean spiny lobster, *Panulirus argus* (Simpson et al., 2017). *Carcinonemertes conanobrieni*, like all described *Carcinonemertes* species is likely a brood parasite that consumes the embryos of its host, though the impact of this relationship is unknown (Simpson et al., 2017). Depending on the species of *Carcinonemertes*, worms may be found on nearly all life stages of their hosts (juveniles, mature adults, and on either sex) or only on gravid females, but no studies looking into the host-use of *C. conanobrieni* on *P. argus* have taken place. *Panulirus argus* is a keystone species in shallow water coral reefs (Behringer & Butler, 2006; Higgs et al. 2016) and makes up one of the most important fisheries in the Greater Caribbean and Gulf of Mexico area. This multimillion-dollar fishery is classified as ranging from fully-exploited to over-exploited across the entirety of its range with approximately 35,000 tons landed in 2014 (FAO, species fact sheet). As a result of both the natural and commercial importance of this lobster, research detailing the variety of marine diseases and pathogens that *P. argus* plays host to, including newly emergent diseases is most relevant (reviewed in Shields et al., 2006 and Shields, 2011). The life life-history of the Caribbean spiny lobster in Florida is well studied (Holthuis 1991, Booth and Phillips 1994, Herrnkind et al. 1994). Juvenile and sub-adult lobsters begin their benthic lives in shallow, near-shore nursery grounds with seagrass meadows and macroalgal beds (Butler and Herrnkind, 2000). Juveniles and sub-adults migrate from near-shore nursery grounds to off-shore reefs where they are attracted to the odors of conspecifics (Childress and Herrnkind 1996, 2001, Ratchford and Eggleston 1998, Nevitt et al. 2000) and are often found sharing crevice shelters (Berrill 1975, Childress and Herrnkind 1997). Once on the reefs, lobster begin to reproduce, with adult females producing at least 2-4 clutches of eggs per

year with larger, older females reproducing earlier and having more clutches per year (Maxwell et al. 2009).

With *P. argus* acting as such an important keystone and commercial species in the Greater Caribbean area, detailing the relationship and the impact that this worm has on such a valuable host is an important step in describing the condition of the spiny lobster fishery in the Florida Keys, and perhaps finding one reason behind the declining lobster landings. Here I will test the hypothesis that the consumption of embryos by this nemertean may influence its host choice. I will explore the host-use pattern and range of *Carcinonemertes conanobrieni* by examining male, non-brooding, and brooding female lobsters collected across the Florida Keys for infection prevalence and intensity. Furthermore, based off of preliminary observations on infected *P. argus*, *C. conanobrieni* may lead to a decrease in reproductive performance in brooding females (Baeza et al., 2016). I will test the hypothesis that infection by this egg-predator leads to a reduction in reproductive performance, and investigate the impact that this worm has on three reproductive performance measures of brooding female lobsters. Finally, I will discuss the implications that infection by this nemertean worm has on the overall health of *P. argus* population and its fishery.

Methods and Materials.

Collection of Sexually Mature, Gravid, and Juvenile Panulirus argus *Specimens.*

Mature male and non-gravid Caribbean spiny lobsters, *Panulirus argus*, were collected by hand (with the aid of a tickle-stick and net) while SCUBA diving from June 9th to July 19th, 2016 from Tennessee Lighthouse offshore coral reef and surrounding patch reefs (5 – 20 m depth) along the

Florida reef tract (Tennessee Lighthouse, approximately 5 km off of Long Key, Florida (24.7707 N, -80.7615 W)).

Gravid *P. argus* were collected in the same locations and time frame as above, but were also collected from July 5th to August 12th, 2017 from the same Tennessee Lighthouse Reef and surroundings while SCUBA diving and additionally from 1 offshore reef (12 m depth) while on a commercial fishing vessel. The commercial vessel was docked at Summerland Key, Florida, and the reef was approximately 3 miles offshore (24.612701 N, -81.446399 W). Gravid specimens were also collected while accounting for lobster embryo stage. The embryos carried by brooding female *P. argus* were classified into four distinct developmental categories based off of physical characteristics. Stage I embryos were recently spawned with a single color throughout and no separation between the yolk and the chorion. Stage II embryos showed the beginnings of cell separation. Stage III embryos showed movement of the yolk inward and thus away from the chorion, eye pigmentation also begins at this stage. Stage IV eggs exhibited elongated eye pigments, evident chromatophores, as well as the formation of a distinct abdomen and other appendages. Stage I and II embryos are classified as early stage, and stage III and IV embryos as late stage (Baeza et al. 2016). We then evenly sampled early and late stage broods to account for the relationship between infection intensity and embryo stage (Baeza et al. 2016).

Juvenile lobsters were collected by hand from June 9th to July 19th, 2016 (with the aid of a tickle-stick and net) while snorkeling over the sand-flats approximately 50-100 m off Long Key, Florida (24.806066 N, -80.800561 W). The collection of all spiny lobsters was possible through a Special Activity License obtained from the Florida Fish and Wildlife Conservation Commission (SAL-15-1674B-SR). All lobsters collected were transported alive in the R/V

Soledad to a temporal laboratory in Long Key, Florida, and maintained alive in 416.5 liter cattle tanks with bubbling aerators until dissection or release.

Determining Infection Status for Male and Non-Gravid Female P. argus.

Prior to dissection of mature and juvenile male and non-gravid female *P. argus*, specimens were placed into individual bags and put into a freezer for ~1 h for euthanization. After ~1 h in the freezer, lobsters were removed and examined for the presence of *Carcinonemertes* worms. First, an initial visual check of the exoskeleton and arthrodial membranes of each lobster was performed to determine the presence or absence of actively roaming or encysted worms with the naked eye. Following this, the cephalothorax and the abdomen were separated and set aside. The carapace of the lobsters was removed (dorsally) and the gill chambers were extracted using forceps. Gills were placed into petri dishes and covered with enough near-shore seawater to allow for full submergence. Next, the pereiopods were removed and set aside. A stereomicroscope or dissecting scope was then used for a more intensive examination of the abdomen, gill lamella, and the arthrodial membranes of the pereiopods. For each lobster inspected, the date and location of capture, carapace length (measured to the nearest 0.5 mm with a caliper), and sex (determined by the presence/absence of extra dactyl on the fifth pereiopod) was recorded. Furthermore, when *Carcinonemertes* worms were found, then the following was also noted: (1) where on the lobster were the worms found. (2) if the worms were found in mucus sheaths, encysted, or free-roaming. (3) if there are any *Carcinonemertes* egg-sacs present.

Determining Infection Status for Gravid Female Panulirus argus.

Prior to the dissection of gravid *P. argus*, specimens were placed into individual containers and then placed into a freezer for ~1 hour for euthanization. After approximately 30 minutes, the lobsters were removed, and all pleopods were cut away. Females were then placed back into the freezer while the eggs were thoroughly searched for the presence of *Carcinonemertes* worms. In order to determine the presence or absence of *Carcinonemertes* in a lobster's brood, 500-1,000 eggs were gently stripped away from each pleopod with the use of fine tip forceps. Lobsters were classified as 'infected' if any of the following was found: 1. adults actively roaming in the egg mass, 2. ensheathed adults, 3. encysted juveniles, 4. *Carcinonemertes* egg cases or larvae, or 5. an abnormally high number of consumed lobster embryos (indicated by empty embryo cases). Conversely, if none of these signs of *Carcinonemertes* infection were found at any point during the eight sub-samples, then the lobster was considered 'uninfected'. Following the determination of infection in the female brood mass, the same protocol as was used above was followed on the rest of the body to determine final infection status.

Collection and Determination of Alternative Host Species.

To determine if a sympatric species of crustacean could also host *Carcinonemertes conanobrieni*, I examined gravid females from two other species - the spotted spiny lobster *Panulirus gutattus*, and the channel-clinging crab *Damithrax spinosissimus*. Only gravid females were examined as a result of preliminary data collected on *P. argus* infection, where if infection were to occur, it would most likely be on gravid females (see results). Gravid females were collected while SCUBA diving from June 9th to August 15th by hand and brought back to the lab alive in the R/V Soledad to a temporal laboratory in Long Key, Florida and maintained alive in aerated 114 L aquaria until dissection. Infection status was determined following the same

protocols used for brooding females of *P. argus.* Collection of these specimens was made possible under a special activity license from FWC (SAL-15-1674B-SR).

Calculating Infection Intensity of Carcinonmertes conanobrieni *on* Panulirus argus.

Through my examinations of gravid female *P. argus*, I found that infection prevalence was nearly 100% (see results). In order to make a comparative analysis detailing the impact of infection of the reproductive health of lobsters, I distinguished between lobsters that had high intensity infections and light intensity infections. To determine what can be considered a 'heavily' infected female lobster and a 'lightly' infected lobster I used two different metrics. First I looked at the number of live, active, and encapsulated worms found in the brood. If more than 10 active worms were found within a count of 4,000 eggs the lobster was considered heavily infected. Then, I took into account the number of consumed embryos and dead embryos that were present in a count of 500 eggs. Consumed embryos were recognized as fully or partially empty egg cases, and dead embryos were recognized by having abnormal size (smaller or larger than surrounding embryos), shape (usually a-symmetrical), and coloration (either a dark brown or a light, milky orange). If more than 10% of the embryos counted were either dead or consumed, the lobster was also considered heavily infected. There were only a few instances where these two metrics did not overlap, and in all three cases it was a result of early stage broods with a high number of encapsulated females, and yet very little brood loss.

Effect of Infection by Carcinonemertes conanobrieni *on Reproductive Performance of* Panulirus argus.

I estimated three different individual-level reproductive performance parameters in 60 brooding female *P. argus* infected with the nemertean parasite *Carcinonemertes conanobrieni*. These parameters include fecundity, reproductive output, and brood mortality. To accomplish this, I first removed all the embryos from the pleopods of the gravid females by gently stripping them away with forceps. Then, five sub-samples of 100 embryos each were isolated from the entire brood mass and dried along with the remaining mass of embryos and the female lobster. Embryos and female lobsters were left to dry for at least 120 hours at 68ºC, and then removed from the oven and weighed to the nearest 0.01 mg with an analytical balance.

Effect of Infection on Fecundity.

Fecundity (total number of embryos produced by an individual female) was calculated with the formula $F = [(((Mass_{embryos}/Average(Mass_{sub1}, Mass_{sub2}, Mass_{sub3}, Mass_{sub4}, Mass_{sub5}))*100)+500)]$; where F = the total number of embryos, $Mass_{embryos}$ = the dry weight of the remaining embryo mass after the five 100 sub-samples were removed, $Mass_{sub\#}$ = the dry weight of one of the embryo subsamples of 100, and the 500 added back in at the end is the total number of embryos removed for the subsamples. The effects that female body size (CL, carapace length), infection status (H, heavily and M, mildly), and egg stage (early (I and II) and late (III and IV)) had on fecundity were tested using a General Linear Model (GLM). JMP Pro 12 was used for this analysis, and the dependent variable was fecundity, the independent variables were egg stage and infection status, and the covariate was carapace length.

Effect of Infection on Reproductive Output.

Next, I estimated reproductive output, a representative measurement for female resource allocation into reproduction, as the ratio between the dry weight of the embryos and the dry weight of the females that carried early stage (I and II) embryos. Embryo dry weight was calculated as the total mass of the five 100 subsamples of embryos plus the mass of all remaining embryos. I first examined the relationship between dry egg mass and female dry mass, using the allometric model $y=a*x^b$ to determine if the relationship was non-linear. In this log-log least squared linear regression, the slope b represents either an estimated rate of increase ($b>1$) or decrease ($b<1$) in resource allocation for reproduction by a unit increase in lobster dry mass. To determine if this relationship deviated from 1 (expected slope of unity) a t-test was used. I then used a GLM to investigate the relationship between female dry mass and infection status on embryo dry mass. JMP Pro 12 was used for this analysis, and embryo mass was set as the dependent variable, female dry mass the covariate, and infection status the categorical independent variable.

Effect of Infection on Brood Mortality.

Lastly, I looked at brood mortality in infected female lobsters. Brood mortality was calculated as a proportion of the number of dead and consumed embryos to live embryos in 500 total embryos counted. Embryos were considered to be dead if they exhibited milky coloration and/or abnormal size and empty embryo cases were characterized as broken/clear egg envelopes where either some or all yolk had been removed (Baeza et al. 2016). A generalized linear model with a normal distribution and an identity link function was then used to test the effect of brood stage and infection status on brood mortality (Warton and Hui, 2011).

Results.

Host-Use Pattern of Carcinonemertes conanobrieni *in* Panulirus argus.

To determine host-use of *Carcinonemertes* worms on male *Panulirus argus* 31 male lobsters were sampled with an even distribution of carapace lengths ranging from 24.90 mm to 92.08 mm. Of these sampled males, there were no signs of *Carcinonemertes* worm infection on the carapace, abdomen, arthrodial membranes, or in the gill chambers.

Thirty non-gravid females were sampled with a distribution of carapace lengths ranging from 12.73 mm to 92.22 mm to determine host-use of *Carcinonemertes* worms on non-gravid female lobsters These females (with the exception of two) also showed no signs of infection by the *Carcinonemertes* worm. In the two instances where adult *Carcinonemertes* worms were found on non-gravid females, they were observed on the abdomen. In both of these instances, there were clear signs that the lobsters were very close to spawning new broods (new sperm patches on the abdomen, and large well-developed ovaries).

To determine the host-use of *Carcinonemertes* worms on gravid *P. argus*, 114 brooding female lobsters with evenly distributed body sizes ranging from 60.24 mm to 87.90 mm were sampled. Of these 114 brooding females sampled, all but 7 showed signs of *Carcinonemertes* infection. Furthermore, of the 107 infected females, all but 6 showed infection that was limited to the brood mass. These remaining 6 females also had one or a few *Carcinonemertes* worms on the abdomen (5 females) or in the gill chamber (1 female). These instances of *C. conanobrieni* worms found outside the brood mass were likely a result of the dissection process. As the pleopods were cut away from the female lobsters, *C. conanobrieni* worms may have been left near the base of the pleopod, or they may have been attached to a clump of embryos that fell away from the pleopod. The one instance of a *C. conanobrieni* worm found within the gill

chamber also had lobster embryos within the gill chamber. It is likely that the worm followed the embryos to the gills.

Host-Use Pattern of Carcinonemertes conanobrieni *in alternative hosts.*

Of the 5 *Panulirus guttatus* and 5 *Damithrax spinosissimus* gravid females collected, none showed signs of infection by *Carcinonemertes conanobrieni*.

Reproductive Performance of Gravid Panulirus argus.

A total of 29 females carrying early stage embryos (I and II) and 31 females carrying late stage embryos (III and IV) were sampled during the summer of 2016, and a total of 4 females carrying early stage embryos and 11 females carrying late stage embryos were collected in the summer of 2017. Of the early stage females sampled, 30 were diagnosed as lightly infected and 3 were diagnosed as heavily infected. Of the sampled late stage females 30 were diagnosed as lightly infected and 12 as heavily infected. The mean (± standard deviation, SD) carapace length of all lobsters sampled was 72.388 ± 6.583 mm and ranged from 60.24 mm to 87.9 mm.

The average (± SD; range) fecundity for all females with early stage embryos (stages I and II) was 219,550.74 (± 70,177.53; 52,189.05 – 397,043.01) embryos lobster $^{-1}$; for all females with late stage embryos average fecundity was 192,880.22 (± 71,288.20; 70,151.52 – 348,267.33) embryos lobster $^{-1}$. Average fecundity for females with early stage embryos and either low or high rates of infection were 214,896.65 (± 70,602.09; 52,189.05 – 397,043.01) and 266,091.60 (± 54,283.37; 232,208.74 – 328,701.93), respectively (Table 3.1, Fig. 3.1). Average fecundity for females with late stage embryos and either low or high rates of infection were 209,818.47 (± 64,648.81; 85,103.62 – 348,267.33) and 145,145.14 (± 70.013.71; 70,151.52 –

287,512.07), respectively (Table 3.1, Fig. 3.1). A general linear model examining the relationship between body size (CL), egg stage (early or late), infection status (low or high), and fecundity did show that both embryo stage (females with early stage embryos had higher fecundity) and carapace length (larger females produced a greater number of eggs) had an effect on fecundity ($F=13.0058$, d.f.= 1, 74, $P= 0.0006$, and $F=130.666$, d.f.= 1, 74, $P<0.0001$, respectively). Interestingly, the general linear model did not show an effect of infection status (high or low) on fecundity ($F=0.0618$, d.f. = 1, 74, $P= 0.8045$). However, the interaction between infection status and egg stage did have a significant impact on fecundity ($F=7.1792$, d.f.= 1, 74, $P= 0.0092$). This significant interaction term can be explained when considering that females with earlier staged embryos tended to have lighter infections than those with later stage embryos. Thus, as embryos develop so too does infection intensity, and as a result, the impact of infection also increases.

Reproductive output (RO) varied between 3.84 and 11.79 % with a mean ± SD of 9.23 % (± 1.79) of lobster dry body mass for all females with early stage embryos (stage I and II). Reproductive output varied between 3.38 and 11.73 % with a mean ± SD of 7.89 % (± 2.12) of lobster dry body mass for all females with late stage embryos (stage III and IV). RO for lobsters with either early or late stage embryos with low infection statuses ranged from 3.84 – 11.79 % with a mean of 8.97 % (± 1.67), and from 3.99 – 11.73 % with a mean of 8.24 % (± 1.79) of lobster dry body mass, respectively (Table 3.1, Fig. 3.1). Lobsters with early or late stage embryos with high infection statuses had a RO that ranged from 10.00 – 11.32 % with a mean or 10.46 % (± 0.75), and 3.38 – 11.40 % with a mean of 6.91 % (± 2.73) of lobster dry body mass, respectively (Table 3.1, Fig. 3.1). A general linear model testing for the effect of embryo developmental stage, infection status, and log corrected female dry body mass on log corrected

reproductive output demonstrated that both female mass (F= 94.8781, d.f.= 1, 74, P<0.0001) and egg stage (F=10.1128, d.f.= 1, 74, P= 0.0022) had a significant impact on reproductive output (i.e. dry mass of embryos). There was no significant effect of infection status on RO detected (F= 0.0885, d.f.= 1, 74, P= 0.767). Interestingly, the interaction between egg stage and infection status was marginally significant (F=3.68, d.f.= 1, 74, P= 0.0591), indicating that as egg stage develops, infection status (intensity) increases.

Brood mortality varied between 0 and 11% with a mean of 1.03% (± 1.86) of lobster embryos for females with early stage embryos (stage I and II). Brood mortality varied between 0 and 64.5% with a mean of 6.74% (± 10.54) of lobster embryos for females with late stage embryos (stage III and IV). Brood mortality for lobsters with either early or late stage embryos with low infection statuses ranged from 0 – 2.04 % with a mean of 0.688 % (± 0.577), and from 0 – 11 % with a mean of 3.78 % (± 3.17) of lobster embryos, respectively (Table 3.1). Lobsters with early or late stage embryos with high infection statuses had brood mortality that ranged from 0.40 – 11 % with a mean or 1.03 % (± 1.86), and 1.83 – 64.5 % with a mean of 6.74 % (± 10.54) of lobster embryos, respectively (Table 3.1). A generalized linear model testing for the effect of embryo developmental stage and infection status on brood mortality demonstrated that infection status (high or low) did play a significant role in brood loss (L-R ChiSquare = 448.881, d.f. = 1, 75, Prob>ChiSq <0.0001). Neither embryo developmental stage (L-R ChiSquare = $2.12*10^{-5}$, d.f. = 1, 75, Prob>ChiSq = 0.9963) nor the interaction between egg stage and developmental stage (L-R ChiSquare = $3.81*10^{-5}$, d.f. = 1, 75, Prob>ChiSq = 0.9951) played a significant role in brood mortality. Interestingly, when removing an outlier point of 64.5% embryo loss, my results which initially showed no difference and infection status then indicated that the interaction between embryo stage and infection status a significant role in brood

mortality (L-R ChiSquare = 365.174, d.f. = 1, 74, Prob>ChiSq <0.0001). Both embryo developmental stage (L-R ChiSquare = $4.35*10^{-5}$, d.f. = 1, 74, Prob>ChiSq = 0.9947) and the interaction between developmental stage and infection statuse (L-R ChiSquare = $1.27*10^{-4}$, d.f. = 1, 74, Prob>ChiSq = 0.991) once again showed no significant impact on brood mortality.

Discussion.

Host use pattern in Carcinonemertes conanobrieni.

Host use in *Carcinonemertes conanobrieni* seems to be affected by host sex, life stage, reproductive stage, as well as location on the host. All male lobsters and all but two non-brooding female lobsters were not infected by this nemertean while all but 7 out of 114 sampled gravid female lobsters were infected by the worm. The above suggests that *C. conanaobrieni* display relatively high host specificity. We also found that in nearly all cases of infection by this nemertean (see results), gravid *P. argus* females only showed signs of infection in their brood masses. In other described species of *Carcinonemertes* worms, host specificity has been shown to vary between highly specific to highly generalistic. *Carcinonemertes carcinophila carcinophila* has been reported as infecting at least 28 species of crustacean host (Humes, 1942; Wickham & Kuris, 1985). *Carcinonemertes carcinophila carcinophila* did not limit its infection to hosts to just the brood masses of gravid females, but could be found on both sexes of host and at all reproductive stages as well (Messick, 1998). Similarly, *Carcinonemertes carcinophila imminuta* has been reported to infected at least 6 different crustacean host species, where the hosts may be of either sex, at any reproductive stage, and on multiple locations of the host's body (Humes, 1942). *Carcinonemertes epialti*, while demonstrating host preference, will infect multiple crab hosts if available (Humes, 1942; Santos et al., 2006). *Carcinonemertes* species

worms are often hard to identify, as they are an example of a group with a cryptic species complex, and have extraordinarily similar morphologies. As a result, the lack of host specificity in some species of *Carcinonemertes* could be a result of this cryptic species complex, rather than true generalist behaviors.

In contrast to the species above, most other described species of *Carcinonemertes* (*C. mitsukurii*, C. *divae*, *C. caissarum*, *C. sebastianensis*, *C. kurisi*, and *C. tasmanica*) have been reported on only a single crab host (though this host fidelity has not been clearly demonstrated) and infection on these hosts is not limited to only brooding females (Humes, 1942; Sadeghian & Santos, 2010). Similarly, *Carcinonemertes errans*, has been shown to be highly species specific when infecting a host and is not limited by host sex or reproductive stage - it has been shown to infect both male and female, adult and juvenile *Cancer magister* (Wickham, 1980). *Carcinonemertes pinnotheridophila* appears to be extremely host specific, and has only been found to infect the brood masses and branchial chambers of female *Pinnixia chaetopterana* (McDermott & Gibson, 1993).

Lastly, *Carcinonemertes wickhami* and *Carcinonemertes australiensis*, both of which are found on panulirid species, have been reported to only infect gravid female lobsters (Campbell et al., 1989; Shields and Kuris, 1990; Shields, 2001). Furthermore, they also limit these infections to the base of the uropods and to the pleopods of egg-bearing females (Campbell et al., 1989; Shields and Kuris, 1990; Shields, 2001). While *C. conanobrieni* is relatively host specific when compared to other species in the genus, it does follow the same pattern of infection as other *Carcinonemertes* species infecting spiny lobsters. Overall, the information above suggests that species of *Carcinonemertes* infecting spiny lobsters are more host specific and appear to be much more specific too with respect to the microhabitat they use in infected hosts (i.e. brood

mass only). Additional studies on the host specificity of *Carcinonemertes* worms are needed, however, before any reliable conclusion on whether or not host phylogeny affects host specificity in this worms.

*Infection Prevalence and Brood mortality in Panulirus argus***.**

Compared to when infection by *Carcinonemertes conanobrieni* was first noted, the prevalence of infection has increased dramatically from 7.4 % (5 out of 68) in 2015 (Baeza et al., 2016) to 93.9 % (107 out 114) for sampled gravid *P. argus* during this study. This increase in prevalence can likely be explained by the modifications to my sampling protocols, rather than an actual change in infection across the population. When not actively searching for signs of infection from *C. conanobrieni*, it is not easily evident, especially with infection intensity being relatively low, and mean brood loss reaching only 6.74% even at high infection intensities. As a result of these difficulties, the new protocol resulted in the number of lobster embryos counted increase by four-fold and infection status was not only determined by the presence of actively roaming adult worms. In general, infection prevalence has been reported for only a few species of described *Carcinonemertes* worms. An investigation into the life history of *C. errans* which is found infecting *Cancer magister*, the Dungeness crab, showed that background prevalence of infection is very high with 98% of sampled hosts being infected (Wickham, 1980). Shields and Kuris (1988) looked at infection prevalence of *C. epialti* on the shore crab *Hemigrapsus oregonensis* in both outbreak and non-outbreak years. In the sampled non-outbreak period *C. epialti* was found at a prevalence of 48% on sampled crabs, while during the outbreak period this prevalence increased to 97% (Shields & Kuris, 1988). *Carcinonemertes australiensis* was found to infect 67% of sampled *Panulirus cygnus* females (Campbell & Gibson, 1989). I do not consider *P.*

argus to be experiencing an outbreak, but that baseline prevalence is likely very high for possible hosts. Still, I cannot discard that prevalence has been increasing in recent years and additional studies might be necessary to determine the full effect that this pathogen with putative major effects has on the reproductive biology and overall health of *P. argus* and the fishery its supports (see below).

Egg mortality, as a total of the number of empty lobster embryos (assumed consumed) and dead embryos in a subsection of the lobster brood, is a measure of the direct impact that this pathogen has on the reproductive ability of its host. *Carcinonemertes* species are all considered egg-predators of their hosts, and their feeding mechanism and rates have been quantified in a couple of species – *C. errans* and *C. epialti* (McDermott, 1976; Wickham, 1979; McDermott & Roe, 1984). I have observed the same suctorial feeding behavior in *C. conanobrieni* as has been previously described in other species (McDermott, 1976; Wickham, 1979; McDermott & Roe, 1984); whereby the nemertean presses its anterior end against a lobster embryo, apparently uses its stylet to make a hole in the egg membrane, and then everts its proboscis into the embryo and begins suck yolk into its body using muscular contractions (pers. obs.) This feeding behavior was observed as leading to a couple of different outcomes. Either the embryo was fed upon until it had its yolk fully consumed, leaving behind an empty embryo case, or its yolk only partially consumed, potentially leading to the misshapen (dead) embryos I observed, adding to the overall brood mortality of the host.

Brood mortality has been reported for only a few species of *Carcinonemertes*. *Carcinonemertes errans*, infecting *C. magister*, contributed to brood mortality that ranged from 7.6% - 63.3% (depending on location) and averaged over 50% of the embryos produced annually (Wickham, 1980). Shields and Kuris (1988) looked at brood loss for the shore crab,

Hemigrapsus oregonensis, infected by *C. epialti* both in outbreak and non-outbreak years. In the sampled non-outbreak period infected female crabs experienced an average brood loss at 5.6%; during the outbreak brood loss for 83% of the measured crabs was 75-100% (Shields & Kuris, 1988). Brood loss experienced by *P. argus* (1.03 – 6.74 %) is similar to that of a non-outbreak year for *H. oregonensis* and for the lower range of infection for *C. magister*.

Implications for the fishery targeting *Panulirus argus*.

The fishery targeting *P. argus* has an estimated value of over $500M US annually, making it one of the most commercially and recreationally important fisheries in the Caribbean (CRFM, 2013). As such, understanding the overall health of the spiny lobster fisheries in the Caribbean is an important step in determining fishery resources and planning management strategies. Increasingly, disease is understood to play a major role in the population dynamics, fisheries, and ecology of marine organisms, especially in lobsters, where there has been a recent rise in the number of diseases infecting them (Behringer et al., 2012). Historically, spiny lobsters have played host to a range of different pathogens, none of which posed any major risk to fisheries (Shields, 2011) however this changed with the discovery of PaV1 (*Panulirus argus* virus 1) (Shields & Behringer, 2004). Since its discovery, it has been estimated that infection prevalence of PaV1 ranges from 0-17% across the Caribbean (Moss et al., 2013). PaV1 is lethal >90% of the time when infecting juvenile lobsters with a carapace length <25 mm, with this percentage sharply dropping once the lobster matures (Butler et al., 2008). While the direct effects of infection by PaV1 are relatively well-known, sub-effects are not as well studied. Pascual-Jimenez et al. (2012) investigated how infection by PaV1 could be altering the physiology, immune response, and immune-competency of *P. argus*, and reported that lobsters infected with

PaV1 also had a 50% higher prevalence of playing host to an opportunistic ciliate infesting the lobster gills at the same time. Indicating that infection by PaV1 could lead to co-infection by another pathogen as a result of a compromised immune response.

Carcinonemertes conanobrieni is yet another example of a newly described pathogen infecting a lobster species. Whether *C. conanobrieni* has been present but unnoticed for decades, or is truly new and has arisen from a compromised immunity, either as a result of PaV1 infection (Pascual-Jimenez et al., 2012) or as a member of a trend of marine hosts that are dealing with warm, eutrophic, and acidic waters where existing pathogens thrive and new diseases emerge (Harvell et al., 1999; 2002) is a question that will require further study. To gain a better understanding of this relationship, investigations describing the ways *C. conanobrieni* interacts with *P. argus* will be needed. One possible option would be to look for a correlation between prevalence of *C. conanobrieni* infection and a lobsters carrying PaV1. Should a correlation exist, and lobsters carrying PaV1 have a higher prevalence of infection, or infections with greater intensities, this could give some insight into whether or not a compromised immunity may have led to this infection.

Regardless, infection by *C. conanobrieni* and the effects that infection have on the reproductive health of the spiny lobster is one more factor that should be taken into consideration when assessing the *P. argus* stock and determining future management strategies. Currently the status of the spiny lobster fishery in the Florida Keys, and across the Caribbean can be classified as "unknown" (Buesa, 2018). This stem from the lack of concrete knowledge surrounding the size of the current lobster populations, size of the breeding stock of lobsters, how well connected lobster populations are, as well as incomplete knowledge about loss of lobster biomass during the fishing season (Buesa, 2018). In one of the latest reports on the Florida spiny lobster fishery,

Buesa (2018) has stated the need for a change in management strategies for the fishery. The new strategies would be a combination of scientific research as well as direct management to better understand what the true level of sustainability of the fishery is. When new management practices are being formed, the ability to input as much information into a possible plan will lead to the most realistic course of action. By integrating a reduction of fecundity for breeding stock (or perhaps the effects of total brood loss that would result from a major outbreak), we can make some inferences about the quality of current as well future lobster stock. This information in turn can be used when making decisions in regards to changes in fishing practices.

Future Directions.

In order to fully understand the impact that the infection by *C. conanobrieni* has on gravid females, additional work will be required. While I have described the direct impact that infection has on the reproductive health of *P. argus*, I have yet to explore if the consequences of infection go beyond reproduction. In general, the relationship between a parasite and its host is intimately linked (Gandon & Michalakis, 2002). This has arisen as a result of the co-evolution of parasites and their hosts, where new host defenses and resulting parasite responses determine the success of infection (Anderson & May, 1982; Gandon & Michalakis, 2002). *Carcinonemertes* spp. are specialized egg predators that are only found on crustacean hosts, and at this broadest context parasite specialization already exists. That *C. conanobrieni* has been found on gravid *P. argus* (which carries its broods outside the body instead of within the abdomen, requiring additional specialization) at a high prevalence without infecting males or non-gravid females, or other sympatric species of crustacean, is an indication that *P. argus* might be its primary host. This being the case, then both host and parasite should display behaviors that indicate ‘awareness’ of

one another. Specific life-history strategies, avoidance/resistance mechanisms, and changes in behaviors are common responses to parasitic infection that should be explored in the future.

Panulirus argus, like many crustaceans with indirect development, displays active parental care whereby the embryos of a brooding lobster are protected by the female by being held for incubation somewhere on/in the body of the female (Phillips & Kittaka, 2000). In a previous investigation into the reproductive strategies of *P. argus*, it was shown that gravid females do exhibit some acts of active parental care towards their broods that go beyond the protection of the embryos on the abdomen (Baeza et al., 2016). Grooming behaviors (flapping, fanning, and pereopod probing) were the most common forms of active parental care in the lobsters I studied. Brood grooming plays multiple roles for the health of the embryo masses, and likely evolved as a mechanism to prevent fouling (through accumulation of sediment, bacteria, algae, fungi, or other organism – Bauer, 1989; Aiken et al., 1985; Silva, 2003; 2007) as well as improve oxygen availability to the developing embryos (Bauer, 1989; Baeza & Fernandez, 2002). While active parental care does occur, it is likely that the time the gravid females spend grooming their broods is impacted by whether or not the brood is infected by *Carcinonemertes* worms or not (i.e. – if females can sense the presence of the egg predator, they will attempt to remove it through cleaning behaviors). This increase in cleaning and brood care behaviors by infected females would indicate awareness of infection, and an attempt to help mitigate or minimize the effects of the *Carcinonemertes* worms. By studying the behaviors of brooding females at varying levels of embryo development and *C. conanobrieni* infection intensities, I will be able to see what impact infection has on brooding behaviors. This, in turn, will indicate whether or not infection by the nemertean has any direct physiological cost (loss of energy through cleaning) on gravid females.

In order to test for this behavioral response in the brooding behaviors of gravid female lobsters, I propose a sampling of infected and non-infected brooding female lobsters evenly distributed across female size and all brood stages off the coast of Long Key, Florida Keys and recording the brooding behavior of these females. Brooding behaviors of the lobsters could be recorded for a period of 24 hours to alleviate the impact of circadian cycles. The specific amount of time that the females spend actively grooming their broods could be recorded during different time blocks of 1 hour that can be randomly chosen (behavior of lobster must be fully visible for the full hour period) and the behaviors quantified. The proportion of time that females spent in each state (amount of time per hour in percentage), as well as frequency and occurrence of each event (number of times per hours) are two possible behaviors that could be used for this. The amount of time that lobsters spend in states as well as the number of grooming events that occur over the course of an hour can be compared between infected and non-infected females across all brood stages. Infected females are expected to increase the amount of time spent performing brooding behaviors compared to non-infected females. Furthermore, time spent brooding should also increase with an increased intensity of infection. The above changes in brooding behavior are likely to limit the negative impacts of infection by removing *C. conanobrieni* worms. However, since there is a correlation between intensity of infection and brood stage, it may be difficult to differentiate between increased brood care as a result of infection or just as a result of later stage broods with higher oxygen requirements. Therefore, sampling and analysis must take this into account, and a comparison between the two sets of data should demonstrate this interaction.

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Table 3.1: Mean (X), standard deviation (SD), and range measurements for gravid *Panulirus argus* reproductive performance parameters (fecundity, reproductive output, and brood mortality) across embryo stage and infection statuses. Fecundity measurements are whole numbers, while reproductive output and brood mortality are presented as percentages.

	Fecundity			Reproductive Output			Brood Mortality		
	X	SD	Range	X	SD	Range	X	SD	Range
Early Stage/High Infection	266,091.60	54,283.37	232,208.74 – 328,701.93	10.46	0.75	10.00 – 11.32	1.03	1.86	0.40 – 11
Early Stage/Low Infection	214,896.65	70,602.09	52,189.05 – 397,043.01	8.97	1.67	3.84 – 11.79	0.688	0.577	0 – 2.04
Late Stage/High Infection	145,145.14	70.013.71	70,151.52 – 287,512.07	6.91	2.73	3.38 – 11.40	6.74	10.54	1.83 – 64.5
Late Stage/Low Infection	209,818.47	64,648.81	85,103.62 – 348,267.33	8.24	1.79	3.99 – 11.73	3.78	3.17	0 – 11

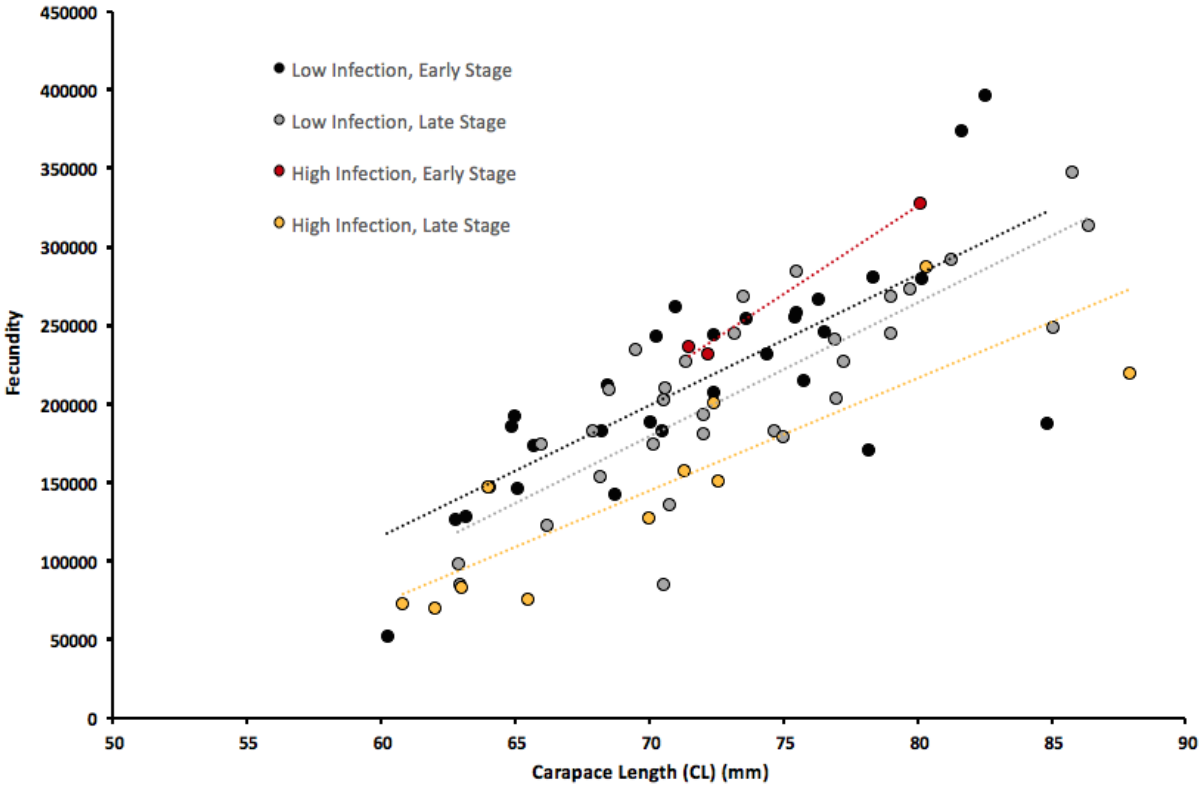

Fig 3.1. Relationship between *P. argus* carapace width and fecundity.

The relationship between female lobster body size and fecundity calculations with both embryo stage (early or late) and infection intensity (low or high) taken into consideration. Embryo stage ($F=13.0058$, d.f.= 1, 74, $P= 0.0006$), female size ($F=130.666$, d.f.= 1, 74, $P<0.0001$), and the interaction between infection intensity and embryo stage ($F=7.1792$, d.f.= 1, 74, $P= 0.0092$) all had an effect on female fecundity estimates.

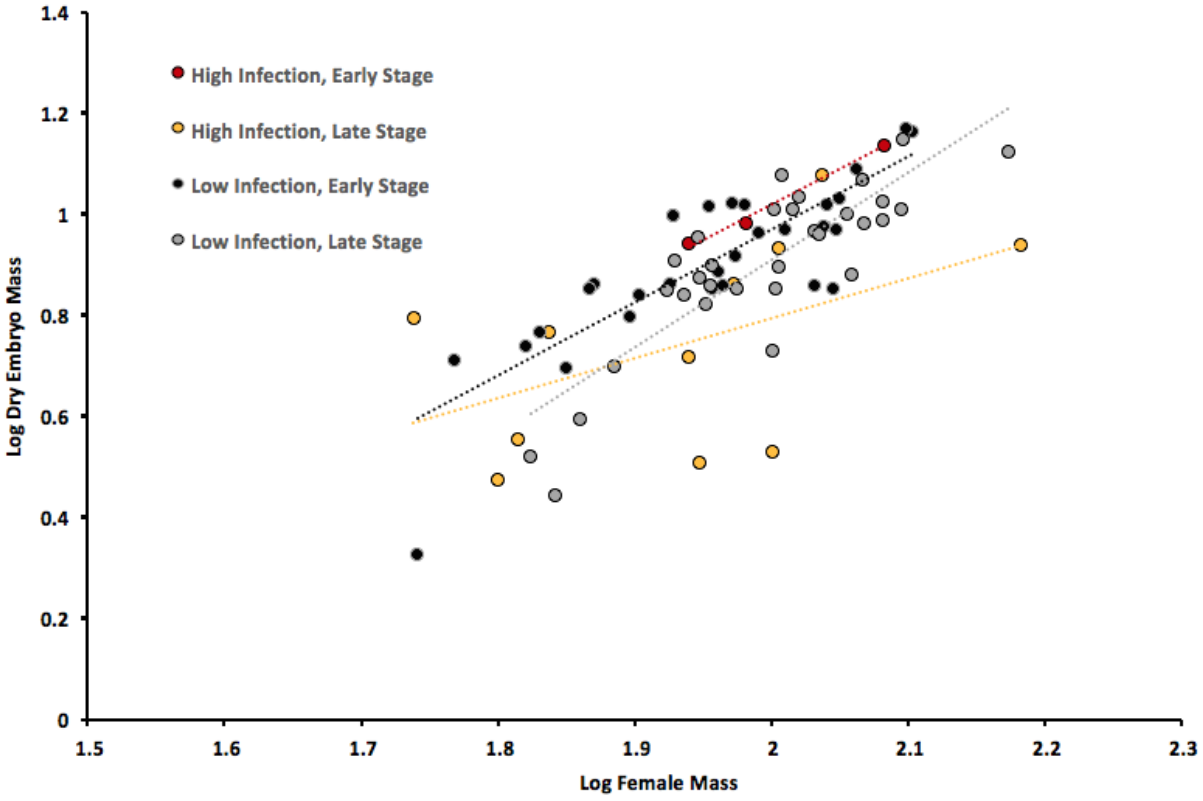

Fig 3.2. Relationship between female lobster body size and reproductive output.

The relationship between female lobster body size and fecundity calculations with both embryo stage (early or late) and infection intensity (low or high) taken into consideration. Embryo stage ($F=10.1128$, d.f.= 1, 74, $P= 0.0022$) and female mass ($F= 94.8781$, d.f.= 1, 74, $P<0.0001$) both had an effect on female reproductive output estimates.