



Salinity and temperature affect the symbiont profile and host condition of Florida USA blue crabs *Callinectes sapidus*

Erin A. Walters^{a,*}, Jamie Bojko^{b,c}, Claire E. Crowley^a, Ryan L. Gandy^a, Charles W. Martin^d, Colin P. Shea^a, Kelly S. Bateman^e, Grant D. Stentiford^e, Donald C. Behringer^{f,g}

^a Florida Fish and Wildlife Research Institute, St. Petersburg, FL 33701, USA

^b National Horizons Centre, Teesside University, Darlington DL1 1HG, United Kingdom

^c School of Health and Life Sciences, Teesside University, Middlesbrough TS1 3BX, United Kingdom

^d Dauphin Island Sea Lab, University of South Alabama, 101 Bienville Blvd, Dauphin Island, AL 36528, USA

^e International Centre of Excellence for Aquatic Animal Health, Centre for Environment Fisheries and Aquaculture Science (CEFAS), The Nothe, Dorset, United Kingdom

^f Emerging Pathogens Institute, University of Florida, Gainesville, FL 32611, USA

^g Fisheries and Aquatic Sciences, University of Florida, Gainesville, FL 32653, USA

ARTICLE INFO

Keywords:

Blue crab
Pathology
Histology
Reflex impairment
Salinity

ABSTRACT

Subtropical Florida blue crabs, *Callinectes sapidus*, exhibit differing life history traits compared to their temperate counterparts, likely influencing symbiont infection dynamics. Little information exists for Florida *C. sapidus* symbiont profiles, their distribution among various habitats, and influence on crab condition. Using histopathology, genomics, and transmission electron microscopy, we describe the first symbiont profiles for Florida *C. sapidus* occupying freshwater to marine habitats. Twelve symbiont groups were identified from 409 crabs including ciliophorans, digenean, microsporidian, Haplosporidia, *Hematodinium* sp., Nematoda, filamentous bacteria, gregarine, *Callinectes sapidus* nudivirus, *Octolasmis* sp., *Cambarincola* sp., and putative microcell. Overall, 78% of *C. sapidus* were documented with one or more symbiont groups demonstrating high infection rates in wild populations. Environmental variables water temperature and salinity explained 48% of the variation in symbiont groups among Florida habitats, and salinity was positively correlated with *C. sapidus* symbiont diversity. This suggests freshwater *C. sapidus* possess fewer symbionts and represent healthier individuals compared to saltwater populations. Crab condition was examined using the reflex action mortality predictor (RAMP) to determine if reflex impairment could be linked to symbiont prevalence. Symbionts were found positively correlated with crab condition, and impaired crabs were more likely to host symbionts, demonstrating symbiont inclusion may boost predictive ability of the RAMP application. The microsporidian symbiont group had a particularly strong effect on *C. sapidus* reflex response, and impairment was on average 1.57 times higher compared to all other symbiont groups. Our findings demonstrate the importance of considering full symbiont profiles and their associations with a spatially and temporally variable environment to fully assess *C. sapidus* population health.

1. Introduction

Callinectes sapidus harbors a diverse array of symbionts throughout its broad latitudinal range, which extends from Nova Scotia to Argentina (Messick, 1998; Shields and Overstreet, 2007; Zhao et al., 2020). The economic importance of *C. sapidus* has prompted many investigations into the diseases affecting fishery landings and marketability (Shield and Overstreet, 2007). Across the United States, landings are at a historic low (Sartwell, 2009; VanderKooy, 2013), possibly due to habitat loss

(Hovel and Lipcius, 2001), disease outbreaks (Messick and Shields, 2000; Shields, 2003), or overfishing. Parasites such as *Hematodinium* sp. and microsporidian can cause severe impacts to the quality and quantity of fishery stocks, yet the impacts from lesser-known symbionts is unclear. Several well-studied symbionts are known to produce stress prior to mortality in crustaceans. The dinoflagellate *Hematodinium perezii* has been documented to cause metabolic and physiological stress to its crustacean host prior to death (Taylor et al., 1996; Stentiford and Shields, 2005). Similarly, microsporidians increase metabolic demand

* Corresponding author.

E-mail address: erin.walters@yahoo.com (E.A. Walters).

<https://doi.org/10.1016/j.jip.2023.107930>

Received 11 January 2023; Received in revised form 15 April 2023; Accepted 1 May 2023

Available online 4 May 2023

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as they consume host metabolic resources (Tsaousis et al., 2008) and create host lethargy (Findley et al., 1981, Stentiford et al., 2013). Fouling organisms such as ectoparasitic gill barnacles (*Octolasmis muerelli*) can disrupt gaseous exchange in heavily infested crabs, possibly leading to mortality (Findley et al., 1981, Gannon and Wheatley, 1992). Additionally, interactive and cumulative effects of multiple symbionts are poorly understood (Bass et al., 2019), especially under the stress of commercial fishing.

Research describing and detailing *C. sapidus* symbiont effects in Florida's sub-tropical climate is lacking, where its life history traits vary markedly in comparison to their temperate counterparts. In its temperate range, water temperatures drop below 10 °C during the winter, prompting crabs to undergo a winter dormancy period (Glandon et al., 2019), while in the subtropics and tropics they are active year long and capable of spawning in all seasons (Hart et al., 2021). Additionally, female crabs in Florida achieve sexual maturity in as little as 6 months due to the protracted warm conditions that accelerate their growth and development (Crowley, 2012, VanderKooy, 2013). Florida's sub-tropical climate permits crabs to reside in freshwater rivers and lakes throughout the year (Tagatz, 1965), influencing symbiont loads differently than their temperature counterparts. To date, its unknown whether these climate-driven differences, combined with life history variability and movement across habitat types through the year, ultimately affect *C. sapidus* symbiont load and diversity and their obligatory stress.

Numerous natural and anthropogenic variables induce stress in marine decapods. Crustacean metabolism is influenced by temperature (Leffler, 1972), respiration rate, and immunological and physiological processes (Shields, 2019). Adult and juvenile *C. sapidus* are less tolerant to water temperature extremes at salinities < 6.8 ppt and > 34 ppt (Tagatz, 1971). Florida's subtropical climate can raise water temperatures over 30 °C for extended time periods, especially in shallow lagoons and estuaries during the summer. These warm conditions increase host susceptibility to pathogens and enhance disease processes that may otherwise be mitigated by lower water temperatures (Huchin-Mian et al., 2018, Shields, 2019). Commercial fishing induces stress when crabs enter crowded traps, and crowded conditions elicit aggressive behavior resulting in injury leading and opportunity for infection and reduced mobility (Welsh and Sizemore, 1985, Guillroy et al. 2001). Further, when traps are brought aboard a commercial vessel, crabs are exposed to handling, desiccation, elevated temperatures, and holding stress (Guillory et al. 2001).

The reflex action mortality predictor (RAMP) method can be used to evaluate stress via reflex responses (Davis, 2010) to estimate delayed mortality among various commercially fished crustaceans (Stoner, 2012, Yochum et al. 2015), including *C. sapidus* (Walters et al., 2022). RAMP research on crustaceans has explored numerous variables that influence the overall probability of delayed mortality in commercially fished species, including intrinsic variables such as molt stage, gender, size, and external variables such as injury (Welsh and Sizemore, 1985), exposure to air (emersion time), and seasonal temperature variations (Rome et al., 2005, Stoner, 2012). The relationship of parasitism on reflex response and its combined association to crustacean stress has not been explored in this important commercially fished species.

In this study, we used histopathology to detail known and novel host-symbiont interactions and diversity across Florida and determine if symbionts affect host reflex response. The information we provide includes a series of new interactions but more importantly provides insight into the potential impact of these symbiotic organisms on host survival, physiology, and persistence in the lucrative *C. sapidus* fishery.

2. Materials and methods

2.1. Collection of *Callinectes sapidus*

Live specimens were collected using commercial fishing gear from

eight different locations throughout Florida including Clapboard Creek Marsh, St. Johns River– Goodbys, St. Johns River –Riverdale, Lake George, offshore of Suwannee Sound, upper Tampa Bay Estuary, lower Tampa Bay Estuary, and the Manatee River (Fig. 1). Collection took place in Goodbys, Riverdale, Lake George, lower Tampa Bay and the Manatee River in the summer months (August– September), and at Clapboard Creek Marsh, Suwannee Sound offshore, upper Tampa Bay and the Manatee River in the winter months (December– February) from August 2019 to February 2021. During each collection trip, commercial fishers hauled traps aboard and shook crabs into a culling box where they were sorted. Those of market grade (legal size and good quality) were retained while all others were discarded. Both market-bound and discard crabs were selected for histopathology analysis as determined by their reflex impairment score (RIS) via a RAMP assessment (Walters et al., 2022). RIS is the total number of reflexes that are absent during a RAMP assessment, where increased loss of reflexes relates to degraded crab condition. To determine the effect of symbionts on RIS, we selected crabs within each RIS category (0–9) and aimed to acquire 75 crabs with low-range RIS (0–2) and 75 crabs with high-range RIS (3–9) during the summer and winter seasons. A RAMP exam was conducted to determine RIS immediately after removal from commercial traps. Nine reflex actions were tested for each crab, including eye retraction, leg retraction, mouth closure, antennule reaction, antennae reaction, joint reaction, chela closure, and chela wave. Additional data recorded for each crab included sex, identified by the abdominal shape (Engel, 1958), carapace width (mm), number of injuries, and wet weight (g). Each crab was then fitted with a wire tag containing a unique identification number and placed in a wooden crate covered with a damp burlap sack while transported alive back to the lab for dissection and tissue collection. A YSI multiparameter probe was used to collect 2–3 salinity (ppt) and bottom water temperature (°C) measurements, which were recorded and averaged for each sampling location.

2.2. Histopathology

Upon arrival at the laboratory, crabs were placed on ice, anesthetized, and dissected immediately to reduce tissue degradation. Crabs were identified by their tag number prior to dissection and any grossly visible macroscopic symbionts within the crabs exoskeleton were recorded (gill dwelling *Cambarincola* sp., grossly observable microsporidian infection in the muscle, and *Octolasmis* sp. infesting the gills). Seven tissue samples were collected from each crab, including gill, heart, epidermis (lining inside dorsal carapace), muscle, gonad, midgut, and hepatopancreas tissue. Tissues were placed in a tissue cassette (compartment size, 28 × 35 mm; Electron Microscopy Sciences, Hatfield, PA) and immediately placed in Davidson's fixative for 48 h on a shaker table to ensure equal tissue fixation. Cassettes were then pulled from Davidson's fixative, rinsed gently in tap water, and transferred to 70% EtOH until processed. Histological processing followed the standard procedures of the Fish and Wildlife Research Institute (FWRI; Florida Fish and Wildlife Conservation Commission) histology department. In brief, tissues were dehydrated by increasing concentrations of ethanol (95% and 100%). Following dehydration, samples were infiltrated with 100% fresh JG-4 resin. The embedded tissue block was sectioned with a Leica RM 2165 microtome 12-mm glass knife to a thickness of 4 µm. Tissue sections were mounted on glass slides and treated with 1% HCl before being stained with hematoxylin and eosin. Stained tissue sections were examined using an Olympus BH-2 compound microscope at 20–100x magnification. The entire tissue section was examined to record parasites and symbionts for each crab. Parasites and symbionts (if relationship with host was unknown or not formerly known) were identified to the lowest possible taxonomic level because species level identity was not always certain using histology alone.

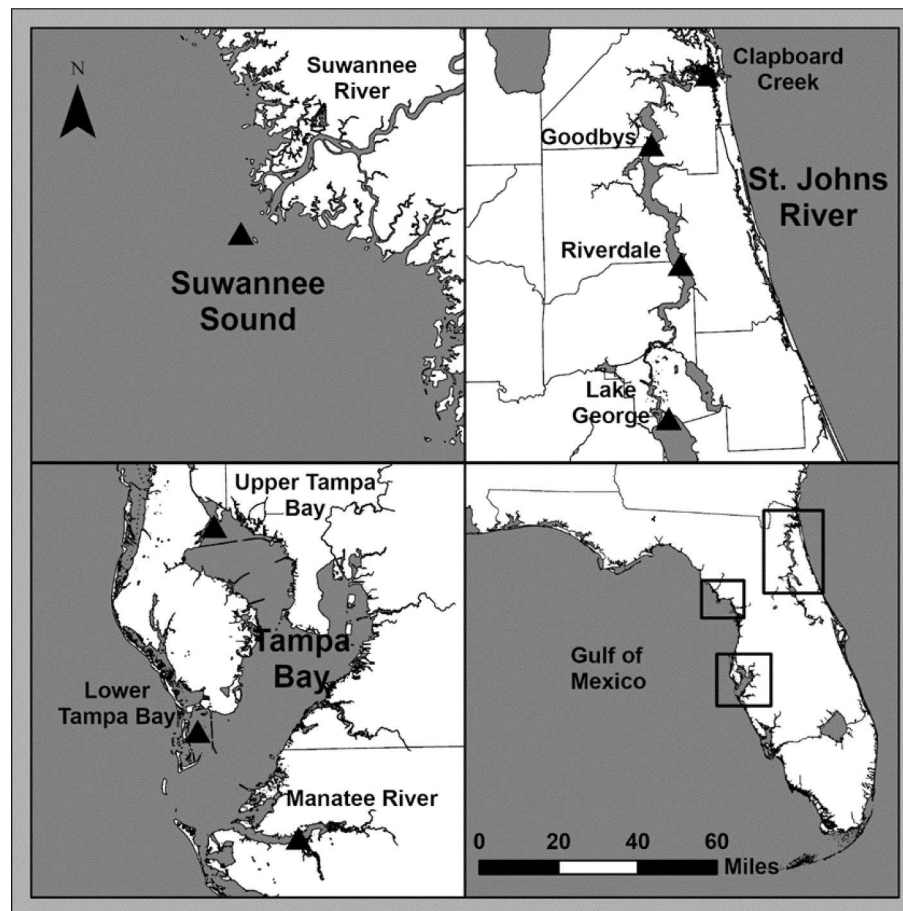


Fig. 1. Map of eight sampling locations (triangles) where crabs were collected on commercial fishing trips.

2.3. Transmission electron microscopy (TEM) from wax-embedded tissues

Wax-embedded blocks that encased tissue sections of putative microcell-like infections were removed with a razor blade. The incised tissue was placed in xylene and then within a rotator to separate the wax from the tissue. Following this step, 100% ethanol was infiltrated to remove the xylene from the tissue entirely and the tissue rehydrated with a series of ethanol to water. Tissue was fixed with a series of reagents including, 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer for 1 h, and then rinsed with 0.1 M sodium cacodylate prior to post-fixation in 1% osmium tetroxide in 0.1 M sodium cacodylate buffer. Sodium cacodylate buffer was used to rinse the tissues prior to undergoing dehydration by series grade acetone and embed with Agar 100 epoxy resin (Agar Scientific, UK). Sections were cut to 1–2 μm and stained with toluidine blue to accurately identify target areas. Target areas were then sectioned to 70–90 nm, stained with Reynolds lead citrate and aqueous uranyl acetate, and mounted on uncoated copper grids (Reynolds, 1963). A JEOL JEM 1400 transmission electron microscope (JEOL USA, Inc, USA) was used to examine target tissue, and an AMT XR80 camera and AMTv602 software were used to obtain digital images and measurements.

2.4. Statistical analyses

2.4.1. Symbiont diversity, variation, and co-occurrence

To quantify *C. sapidus* symbiont diversity, a Simpson Diversity Index (SDI) was calculated for each location where crabs were collected. The SDI is suitable for determining diversity and relative abundance of symbionts (Magurran, 1988). Crabs total number of symbiont taxa in

each symbiont taxonomic group were tallied to calculate an SDI for each location. The SDI equation is defined as $D = 1/(\sum n(n-1)/N(N-1))$, where D is the Simpson diversity index, n is the number of individuals with a single symbiont species, and N is the total number of symbiont taxa documented. The SDI ranges from 0 to 1, where values close to 0 represent reduced symbiont taxonomic diversity, and values close to 1 represent increased symbiont taxonomic diversity. Locations that were sampled in both the summer and winter seasons were analyzed separately to account for the potential symbiont diversity differences associated with temperature. Sampling all eight locations in each season was not feasible given sampling was contingent on commercial fishers trapping patterns who frequently move their traps to seek the greatest catch. Pearson's correlations were used to determine if the salinity and seasonal temperature gradients had significant ($p < 0.05$) correlations with the diversity of symbionts. Diversity analysis for SDI was carried out through the Vegan package in R version 4.0.3 (R Core Team, 2020).

Ordination analysis was used to determine how environmental variables (Özer and Kirca, 2015; Chen et al., 2019) water temperature, salinity, location, and season affect symbiont variation among different Florida habitats. Data were aggregated by collection date ($n = 20$), and species-level data included 10 symbiont groups (dependent variables), and environmental data included salinity (ppt) and water temperature ($^{\circ}\text{C}$) (independent variables). Collinear and insignificant predictor variables were not included in final model selection. Two symbiont groups (gregarine and Nematoda) were removed due to low prevalence (≤ 5 individuals), not meeting relative frequency criteria, and contributing little interpretative value (Gauch and Gauch, 1982). Prior to the implementation of RDA, data were normalized based on the Euclidean distance (Legendre and Gallagher, 2001). Following the RDA, an ANOVA was applied to the axis and the environmental terms to

determine if environmental variables were significantly related to symbiont group occurrence. RDA analysis was carried out using the Biodiversity R package and all statistical analyses were performed with R version 4.0.3 (R Core Team, 2020).

To explore symbiont coinfection, we classified pairwise co-occurrence of symbionts on *C. sapidus* as either positive, negative, or random (i.e., no significance) using a probabilistic co-occurrence algorithm that was implemented in the R package “cooccur” (Veech and Peres-Neto, 2013; Griffith et al., 2016). Data were aggregated for each crab and symbionts were recorded as present or absent. The probabilistic model calculates the expected and observed frequencies of co-occurrence among each pair of species (here, symbionts on crabs), where expected frequency is calculated based on the assumption that the distribution of each species (symbiont) is random and independent of the other. The algorithm returns the probabilities that a more extreme (either low or high) value of co-occurrence could have been obtained by chance. Default threshold settings were used such that species pairs with insufficient data (defined as less than one expected co-occurrence) were excluded from the analysis.

2.4.2. Symbiont taxonomic richness

The word “symbiont” is used to broadly describe organisms (parasitic, commensal, and mutualistic) that form a close or intimate relationship with a host organism (Overstreet, 1978). For the purposes of this paper, symbiont includes parasitic, commensal, and mutualistic associations. Moreover, the precise relationship between some of the symbionts found and their *C. sapidus* host is unknown. Symbionts were recorded from all tissue samples for each crab via microscopic and gross examination during dissection. They were identified to the lowest taxonomic level and placed into groups (e.g., Ciliophora, Digenea). Each crab was assigned a symbiont taxonomic richness score (STR) quantified as the sum of different symbiont taxonomic groups for each individual (i.e., crab noted with *Hematodinium* sp. and ciliophorans had a STR score equal to 2). To determine the relationship between a crabs RIS and STR, a Pearson’s correlation and a Poisson regression model were used. The RIS was the response variable and STR the predictor variable. Pearson correlation calculations and Poisson regression modeling were conducted in R v4.0.3 (R Core Team, 2020).

2.4.3. Symbiont effect on host reflex response

As noted above, for many symbiotic organisms the impact on the host is uncertain. To determine the influence of individual symbiont groups on host reflex response, a negative binomial regression was used to model *C. sapidus* RIS as a function of the presence of each symbiont group. All possible symbiont groups associated with each sample (crab) were expressed as separate binary variables, where “1” indicated that a symbiont was present on a crab and “0” otherwise. Each model included only a single symbiont’s presence or absence as a predictor variable. Akaike’s information criterion (AIC; Akaike, 1973) with a small-sample bias adjustment (AICc; Hurvich and Tsai, 1989) was used to identify which symbiont groups explained RIS, where lower-ranking models (lower AICc) indicate better fit. AIC weights (Burnham and Anderson, 2002) were then used to assess the relative support for each species being the best predictor of *C. sapidus* RIS. We considered symbiont groups important predictors of RIS if the 95% confidence interval of their estimate did not overlap zero. Symbiont groups with < 15 occurrences were excluded from the analysis to avoid making inferences based on small sample size. The negative binomial regression modeling was conducted in R v4.0.3 (R Core Team, 2020).

3. Results

3.1. Host information, symbiont prevalence, and histopathology

A total of 409 crabs from eight different locations in Florida (Fig. 1) were assessed for RIS and symbiont frequency. Male (n = 351), mature

female (n = 51), and immature female (n = 7) crabs had a mean carapace width $136.03 \text{ mm} \pm 14.32 \text{ SD}$ and range of 71–171 mm. The majority (77%) of crabs had a $\text{RIS} \leq 2$ and the number of crabs in each RIS category decreased with increasing RIS score (Fig. 2).

Histological evaluation revealed 12 symbiont groups, including ciliophorans (*Epistylis* sp., *Acineta* sp., *Lagenophrys callinectes*), gooseneck barnacles (*Octolasmis* sp.), microsporidian, *Callinectes sapidus* nudivirus (CsNV), Digenea, Haplosporidia, *Cambarincola* sp., *Hematodinium* sp., Nematoda, filamentous bacteria, putative microcell-like parasites, and gregarines (Apicomplexa). The apparent prevalence (the proportion of infected crabs) of specific symbionts was ciliophorans (47.2%), *Octolasmis* sp. (18.6%), *Cambarincola* sp. (17.6%), filamentous bacteria (16.6%), Digenea (14.4%), microsporidian (4.4%), putative microcell-like parasites (3.9%), Haplosporidia (3.4%), CsNV (2.2%), *Hematodinium* sp. (2.0%), Nematoda (1.2%), and gregarines (0.2%). The highest range of STR groups hosted by any given *C. sapidus* was six, and most (78%) hosted one or more STR groups (Fig. 2). Additionally, 38% hosted two or more STR groups (Fig. 2). It should be noted that prevalence of symbionts is based from a small tissue sample, and the remaining 22% of crabs that were found with no symbionts is likely an underrepresentation.

Stalked or non-stalked ciliophorans of the gill or other extremities were observed in histological sections, presumably belonging to *Epistylis*, *Acineta*, and *Lagenophrys* genera. *Epistylis* were vase-like to round-shaped zooids attached via a stalk, often in colonies (Fig. 3A). *Acineta* were square shaped lorica with pronounced stoma that were cemented to the host tissue by a disk-shaped holdfast (Fig. 3A). *Lagenophrys callinectes* was defined by its round (dorsal and ventral views) transparent lorica that encases the ciliates internal structures (Couch 1967) (Fig. 3B, C). Depending on plane of view, the lips of the buccal aperture were also distinguishable. Crabs could be colonized by all three ciliates. One crab was noted with an unidentified ciliate in the hemal sinus of gill lamellae and in the epithelium (Fig. 3D). These ciliates were cylindrical to round and approximately 15–25 μm in width, with a deeply staining basophilic macronucleus, a micronucleus, and numerous kineties, resembling infections akin to scuticociliates (Miller et al., 2013; Small et al., 2013). Occasionally, stalked ciliophorans were found at a high burden, with several hundred present in gill sections.

Octolasmis sp. (ectoparasitic gill barnacles) were observed macroscopically, occasionally at high infestation (>50 *Octolasmis* sp.), on the external gill surface and the underside of the upper carapace (Fig. 3E). Barnacles in histological section were large and easily defined by a distinctive peduncle (stalk that is cemented to host tissue) and capitulum that could also be observed in histological sections (Fig. 3E). The barnacles spermatogonia (capitulum), eggs (mantle), and ovaries (peduncle) were discernible in various histologic sections.

Cambarincola sp. were observed macroscopically in the gills and within the external surfaces of the gill chamber of nearly all freshwater (<3 ppt) crabs. The annelid was identified based on its small 3-mm length and light pink color, 15 total body segments, a full-length alimentary system, and definable head with jaws and a mouth (Gelder and Messick, 2006) (Fig. 3F). Histopathology revealed the branchiobdellid ultrastructure and indicated no visible host immune response (Fig. 3F).

Nematoda were observed in the host muscle tissue (Fig. 4B) and in between gill lamellae of female crabs (Fig. 4A). Nematodes in general were identified by their round body cavity in cross section, surrounded by muscle tissue encasing the alimentary tract (esophagus and intestine). The several nematodes in between gill filaments of female crabs were encased in a clear mucous sheath, likely belonging to the genus *Carcinonemertes* (Humes, 1942). Several *Carcinonemertes* were common in a single histologic section, suggesting that in some hosts high burden is likely. Despite the potential for high burden, no host immune response was visible.

Digeneans were encysted (metacercariae), presenting a thickened outer membrane and located in connective tissues surrounding the

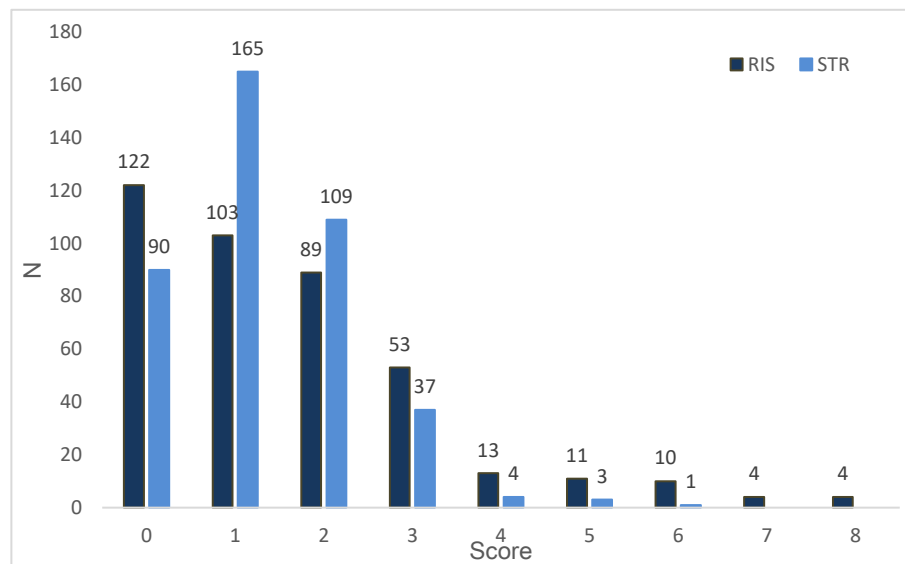


Fig. 2. Number of crabs collected with each Reflex Impairment Score (RIS) and symbiont taxonomic richness score (STR) across all sampling sites between August 2019 and February 2021.

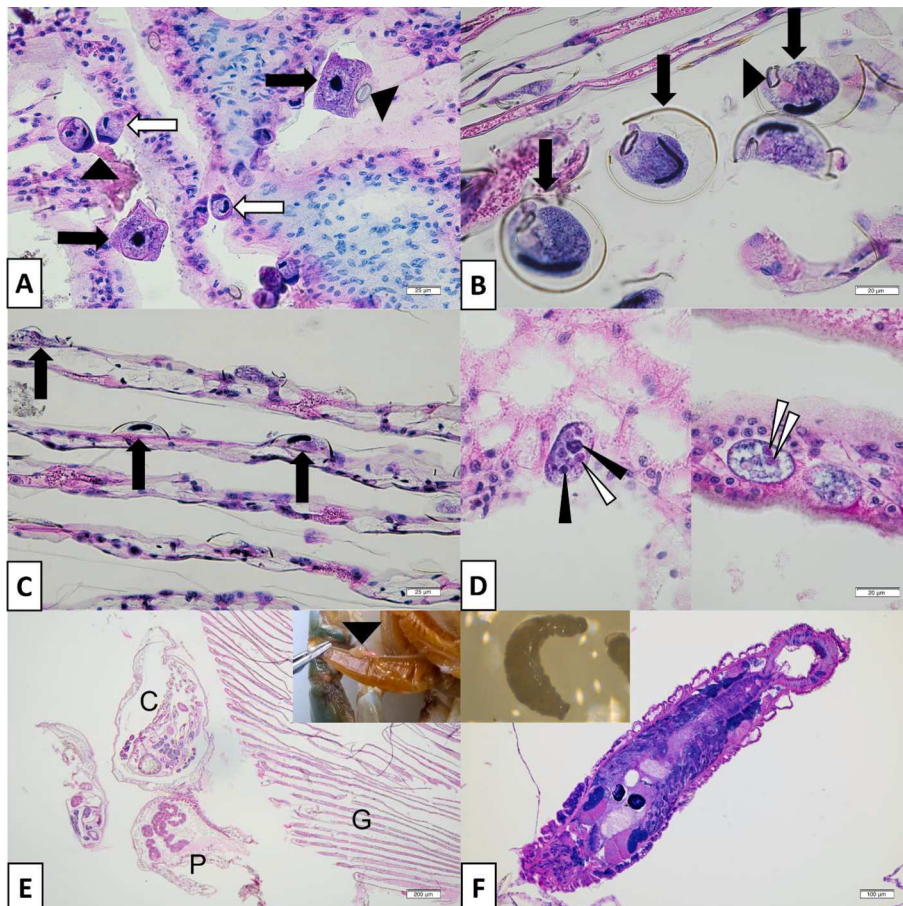


Fig. 3. Various gill infections of *Callinectes sapidus* from Florida. A) Peritrich ciliate forms including, *Epistylis* (white arrow) attached to gill lamellae by branched stalks (black arrowhead) and *Acineta* (black arrow) attached by a disc-shaped holdfast (black arrowhead). B) Unattached loricate ciliate *Lagenophrys callinectes* displaying buccal aperture (black arrowhead) and U-shaped basophilic macro nucleus. C) *Lagenophrys callinectes* attached to gill lamellae in lateral view. D) Ciliate in the hemal sinus of gill lamellae and cuticle displaying macronucleus (black arrow), micronucleus (black arrow) and numerous kineties (white arrow). E) Gooseneck barnacle (black arrowhead) attached to gill lamellae seen macroscopically during dissection and histological, ventral view of the barnacles capitulum (C) and peduncle (P) sections among gills (G). F) Ventral section of *Cambarincola* displaying full length alimentary tract and distinct head.

hepatopancreatic tubules, the tegmental glands of gill tissue and the midgut (Fig. 4C). In some cases, metacercariae had numerous basophilic hemocytes surrounding the encysted parasite, suggesting immune recognition by the host. The metacercarial sucker and portions of its alimentary tract were visible in section and the thickness of the cyst wall varied but was not quantified.

Gregarines were observed in the gut lumen of one host (Fig. 4D). Taxonomic status could not be established, although these parasites contained a large and elongate deutomerite with a distinctive epimerite and pronounced nucleus. Infection based on the one case did not cause any apparent host immune response.

The dinoflagellate parasite *Hematodinium* sp. was observed in the

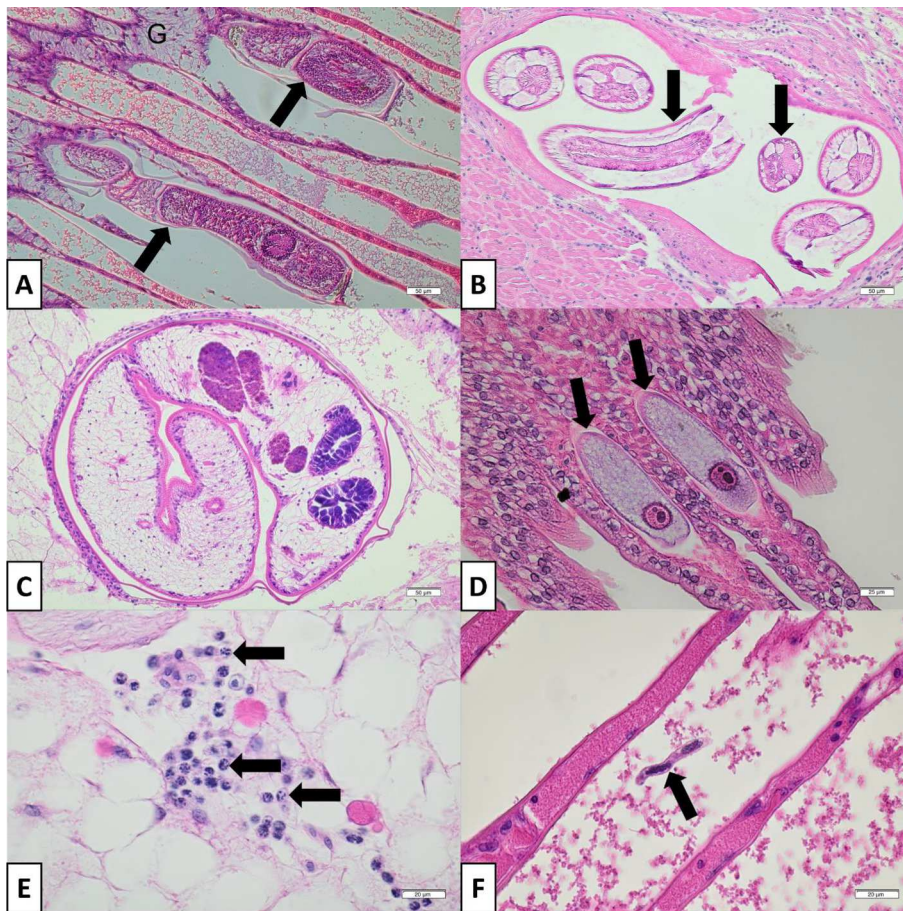


Fig. 4. Nematoda and protozoan infections of *Callinectes sapidus* from Florida. A) Nematode belonging to genus *Carcinonemertes* (black arrow) in between gill (G) lamellae. B) Cross section of a nematode in muscle displaying round body and housing body wall and digestive tract. C) Encysted digenea metacercariae in tegmental gland of gill. D) Unidentified gregarines (black arrow) of the midgut epithelium. E) Small and single multinucleate stages (black arrow) of *Hematodinium* sp. within the epithelial cuticle. F) Advanced elongate *Hematodinium* sp. (black arrow) stage of the gills.

hemolymph surrounding the gills, muscle, heart, hepatopancreas, and epithelial tissues. Various stages of the parasite were noted, including uninucleate, multinucleate, and the elongate plasmodial stages (Fig. 4E, F). Often the parasite was found at high burden; however, no melanization response was visible in histological section.

Microsporidian spores were noted in the skeletal muscle and in some cases the myocardial portions of the heart and inner epithelial lining. Parasites were identifiable by their light-to-dark basophilic staining, most often in the host cell cytoplasm, such as in the muscle sarcolemma (Fig. 5A,B). Dense groupings of the parasite life stages were often found at high burden in infected host tissue growing along muscle fibers and replacing healthy muscle with microsporidian spores whilst leaving adjacent muscle intact.

A putative microcell-like parasitic infection was documented in crabs from the lower Tampa Bay estuary during the summer sampling season of 2019. The infection was noted in several tissue types, including the gonad, tegmental gland of the gill stem, and heart (Fig. 5C). Unicellular microcells were present in epithelial tissue, and in some cases the free plasmodial form was present. TEM detail from wax-embedded tissue revealed spherical uninucleate and multinucleate cells measuring 1–2.5 μm , resembling the mikrocytids (Fig. 6a–b). In each of these tissues small nucleated cells were noted in the cytoplasm of the infected organ or tissue type. In Fig. 5C, we highlight the epithelial cells of the vas deferens (male gonad), where small basophilic nuclei with their own small but evident cytoplasm are visible in the host cell cytoplasm (black arrows). Based on the pathology of infected epithelial cells in the vas deferens and the parasites' physiology, the parasite was consistent with previous descriptions of mikrocytids; such as *Paramikrocytos* from *Cancer pagurus*, which infects the epithelia of the antennal gland. We are somewhat restricted with tissue re-processing quality, and list this observation as putative until greater molecular and ultrastructural detail

is available.

Hyperparasitic Haplosporidia (*Urosporidium crescens*) were detected with encysted digenean metacercariae infecting host tissues. The spores appeared dark brown to black in histological section and infected only the encysted metacercaria (Fig. 5D), leaving the surrounding host tissue visibly uninfected. The crab host did appear to trigger a visible immunological response to infected metacercaria.

Filamentous bacteria were basophilic and strand-like attached (by a basal holdfast) to the lumen of hepatopancreatic tubules and in a few cases within the midgut (Fig. 5E). The filamentous bacteria extended into the open hepatopancreatic lumen. No host immune response was visible, but in some hosts, hepatopancreatic epithelia were seen sloughing at the attachment site.

Nine crabs (2%) were recorded with the *Callinectes sapidus* nudivirus (CsNV) infection using histopathology. CsNV infections were recorded from crabs where the salinity ranged from 0.5 to 29.1 ppt, in both summer and winter seasons. CsNV was observed as an intranuclear infection in host hepatopancreatic epithelia, where the infected cell exhibits an enlarged nucleus with an eosinophilic/basophilic viroplasm causing marginalized chromatin Bojko et al. (2022) (Fig. 5F).

3.2. Symbiont diversity, variation, and co-occurrence

Symbiont diversity represented by the SDI, were correlated with season and environmental variables. Nine SDI measures were calculated, one for each of the seven locations sampled during a season, and two for the Manatee River location that was sampled in both the summer and winter seasons (Fig. 7). The SDI for *C. sapidus* symbionts at each sampling location was significantly correlated with mean salinity (Pearson's correlation, $r = 0.897$; $df = 7$; $p = 0.0010$). As salinity increased, the diversity of symbionts increased (Fig. 7). The lower

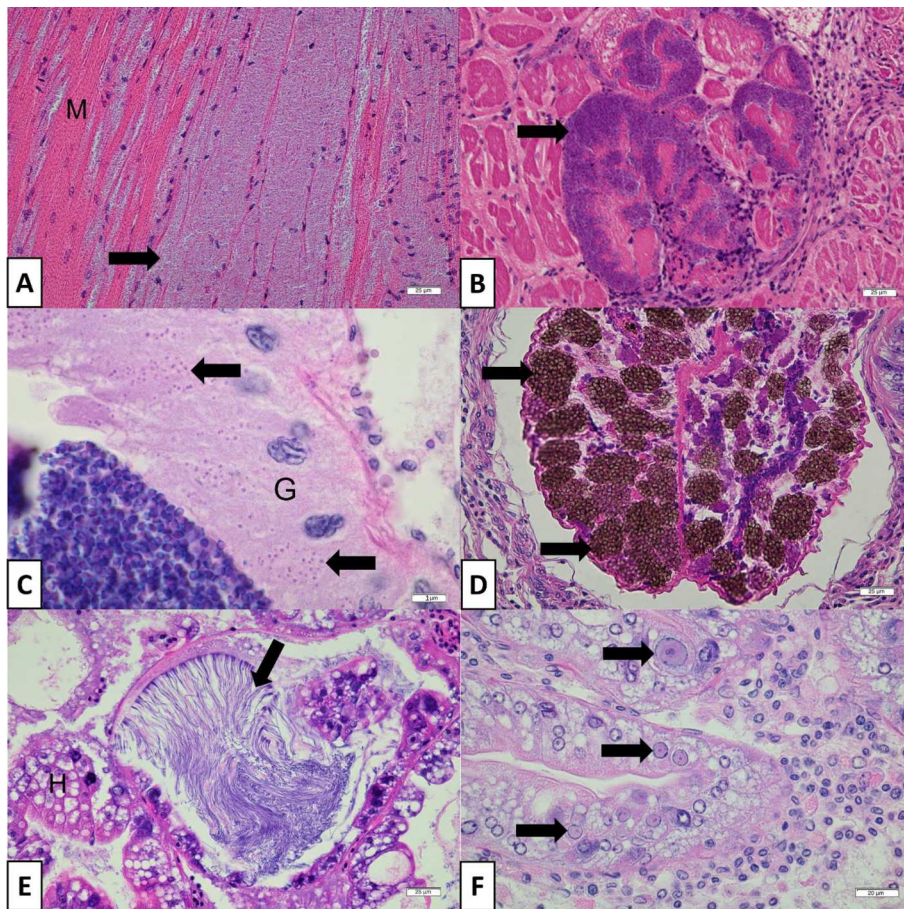


Fig. 5. Various infections of *Callinectes sapidus* from Florida. A) Microsporidian spores (black arrow) displacing healthy muscle tissue. B) Deep basophilic stained microsporidian spores (black arrow) of the heart tissue. C) Unicellular microcell infection (black arrow) of the vas deferens epithelial tissue. D) Haplosporidian, *Urosporidium crescens* (black arrow) hyperparasitizing a digenean of the hepatopancreas tissue. E) Unidentified filamentous bacteria (black arrow) designated by basophilic strand-like filaments attached to the lumen of hepatopancreas tubule. F) CsNV showing enlarged hepatopancreatocytes with emarginated chromatin (black arrows) of infected cells.

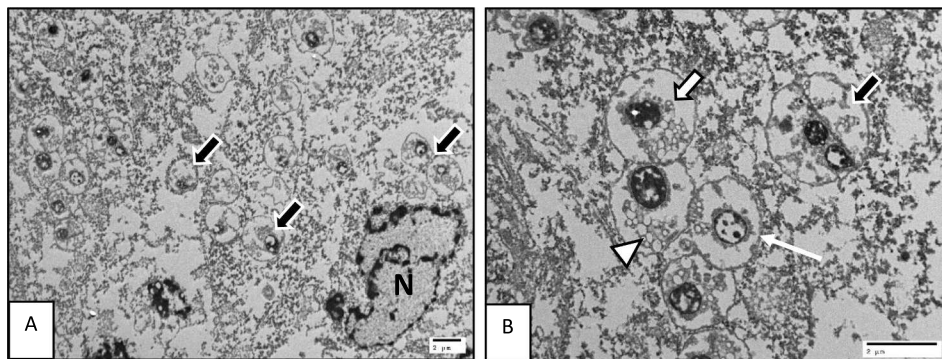


Fig. 6. Transmission electron microscopy of putative microcell infection of *Callinectes sapidus* from Florida. A) Spherical cells (arrows) measuring 1–2.5 μm were observed in the cytoplasm of host cells adjacent to host cell nucleus (N). B) Uninucleate (white arrow) and binucleate stages (black arrow) were observed, surrounded by electron lucent vesicles (arrowhead), all contained within a clear membrane (line arrow). Scale bars = 2 μm .

Tampa Bay location had the greatest mean salinity (30.062 ppt) and SDI (0.769) where 9 of the 12 symbiont groups were documented. Conversely, the lowest mean salinity (0.553 ppt) was at Riverdale, St. Johns River, and coincided with the lowest SDI (0.458), where 4 of the 12 symbiont groups were documented. Mean water temperature and symbiont diversity were not significantly correlated ($r = -0.320$; $df = 7$; $p = 0.401$).

The most common STR group across all crabs and locations were ciliophorans (47.2%) and least common were the gregarines (>1%) and nematodes (>2%) (Table 1). *Cambarincola* sp. were present in habitats with < 3 ppt salinity (Lake George, Goodbys and Riverdale, St. Johns River). Of the 74 crabs collected from freshwater systems, 98% had

Cambarincola sp. on their gills and gill chambers. Comparatively, Haplosporidia, *Hematodinium* sp., putative microcell-like infections, and nematodes were present only in habitats with >26 ppt salinity (Fig. 8).

Symbiont variability among locations was influenced by environmental parameters. Environmental salinity and temperature explained almost half of the symbiont variation under constrained ordination (47.9%). ANOVAs demonstrated that both environmental variables (salinity and temperature) were significantly related to symbiont prevalence ($p < 0.001$). Additionally, RDA accounted for 100% of the total variance and was significant for both the first ($F = 10.86$; $p = 0.001$; 999 permutations) and second ($F = 5.74$; $p = 0.002$; 999 permutations) axes within the model. Those infected with *Cambarincola* sp. received the

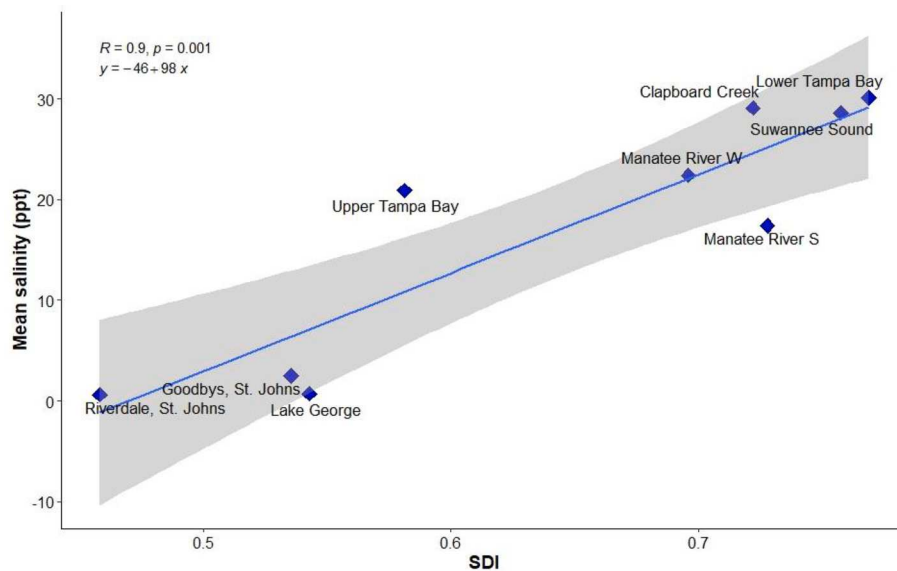


Fig. 7. Diversity of blue crab symbionts (measured by Simpson Diversity index [SDI]) and mean salinity (ppt) for each site (diamonds) sampled. Manatee River summer (Manatee River S) and Manatee River winter (Manatee River W) seasons were analyzed separately to account for seasonal variability. Linear regression of symbiont diversity and mean salinity at each site and associated 95% confidence intervals (grey band). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1

Total number (N) of microorganism groups, Ciliophora spp., Digenea, CsNV, filamentous bacteria, *Octolasmis* sp., *Hematodinium* sp., *Cambarincola* sp., microsporidian, Haplosporidia, microcell-like, Nematoda, gregarine, total crab count (crab N), crab mean reflex impairment score (Mean RIS), crab mean parasite score (Mean PS), Mean Salinity, collection season (Summer and Winter) for each of the eight sampling locations (Clapboard Creek, Goodbys St. Johns, Riverdale St. Johns, Lake George, Suwannee Sound, Upper Tampa Bay, Lower Tampa Bay and Manatee River).

	Clapboard Creek	Goodbys, St. Johns	Riverdale, St. Johns	Lake George	Suwannee Sound	Upper Tampa Bay	Lower Tampa Bay	Manatee River
Ciliophora sp. N	17	12	22	8	6	20	61	47
Digenea N	1	1	1		11	4	26	15
CsNV N	4		1	1	1	2		
Filamentous bacteria N	4				1	8	2	53
<i>Octolasmis</i> sp. N	2				2		38	34
<i>Hematodinium</i> N	5				1		1	1
<i>Cambarincola</i> sp. N		13	49	10				
Microsporidian N					1		10	7
Haplosporidia N					5		9	
Microcell N							16	
Nematoda N	3						2	
Gregarine N								1
Crab N	47	14	49	11	21	37	138	92
Mean RIS	1.128	1.786	1.673	0.273	1.000	0.919	2.123	1.696
Mean PS	0.72	1.857	1.49	1.727	1.29	0.919	1.20	1.65
Mean Salinity	29.119	2.500	0.553	0.700	28.600	20.851	30.062	19.349
Summer		✓	✓	✓			✓	✓
Winter	✓				✓	✓		✓

highest score on the positive side of the first axis, highlighting the symbionts preference to decreased salinities (Fig. 9). *Hematodinium* sp. preferred elevated salinities (≥ 21 ppt) in Florida's mild winters (88% documented below 20°C) primarily at the Clapboard Creek location (Fig. 9). A seasonal predictor variable was not used because of the collinearity with water temperature (0.94) and location, which lacked significance in the model.

Co-occurrence analysis identified seven positive and six negative co-occurrence relationships among symbiont groups (Fig. 10). Of 78 species-pair combinations, 42 pairs (58.5%) were removed from the analysis because the expected co-occurrence value was < 1 , leaving 36 pairs for analysis. Symbiont groups excluded from analysis included *Hematodinium* sp., gregarines, and nematodes since they did not achieve relative frequency criteria. Significant relationships included ciliophorans and digeneans ($p = 0.030$), microsporidians and digeneans ($p = 0.032$), microsporidians and filamentous bacteria ($p = 0.008$), *Octolasmis* sp. and digeneans ($p < 0.0001$), *Octolasmis* sp. and filamentous bacteria ($p = 0.026$), *Octolasmis* sp. and microsporidians ($p = 0.033$),

and haplosporidians and digeneans ($p = 0.000$). Negative relationships included ciliophorans and CsNV ($p = 0.003$), *Cambarincola* sp. and microsporidians ($p = 0.028$), *Cambarincola* sp. and putative microcells ($p = 0.035$), *Cambarincola* sp. and digeneans ($p = 0.001$), *Cambarincola* sp. and *Octolasmis* sp. ($p < 0.0001$), and *Cambarincola* sp. and filamentous bacteria ($p < 0.0001$) (Fig. 10).

3.3. Symbiont effect on host reflex response

The relationship of sub-tropical *C. sapidus* symbionts on RIS was found positively associated. A Pearson's correlation identified a significant positive relationship between RIS and the STR score (0.19; $p < 0.001$). Preliminary analysis indicated under-dispersion (i.e., RIS counts were less variable than expected under a conventional Poisson distribution), and therefore a Conway-Maxwell Poisson was used (Huang, 2017). The parameter estimate associated with STR (estimate = 0.175 and standard error = 0.046) indicated a positive relationship between RIS and STR, with RIS increasing by 1.19 times, on average, for every 1

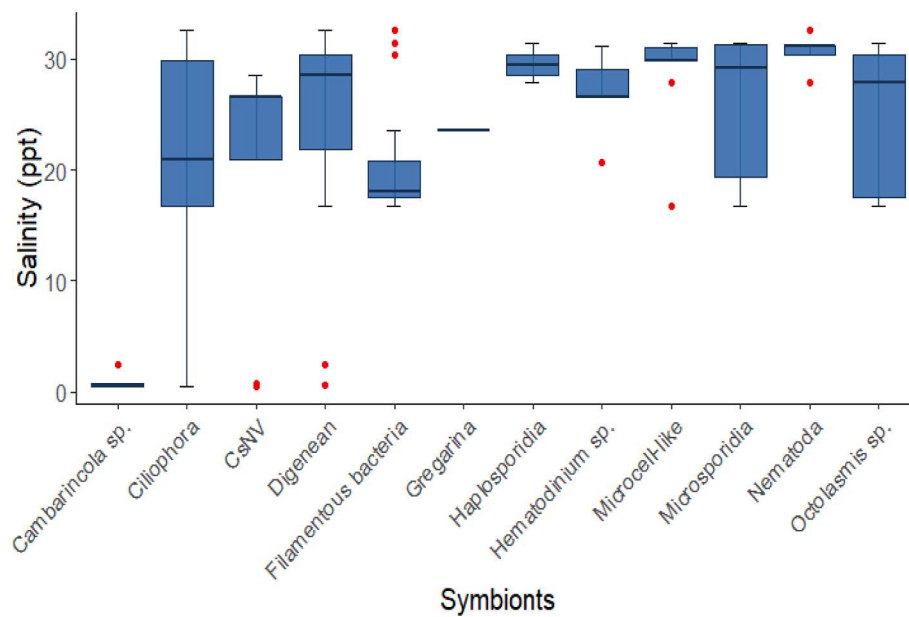


Fig. 8. Box plots of symbiont taxonomic richness (STR) groups and their salinity range. The blue boxes represent the interquartile range (25th–75th percentile), the black line inside the boxes represents the median, and the whiskers represent the maximum and minimum salinity values for each symbiont group. The individual red points represent the outliers. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

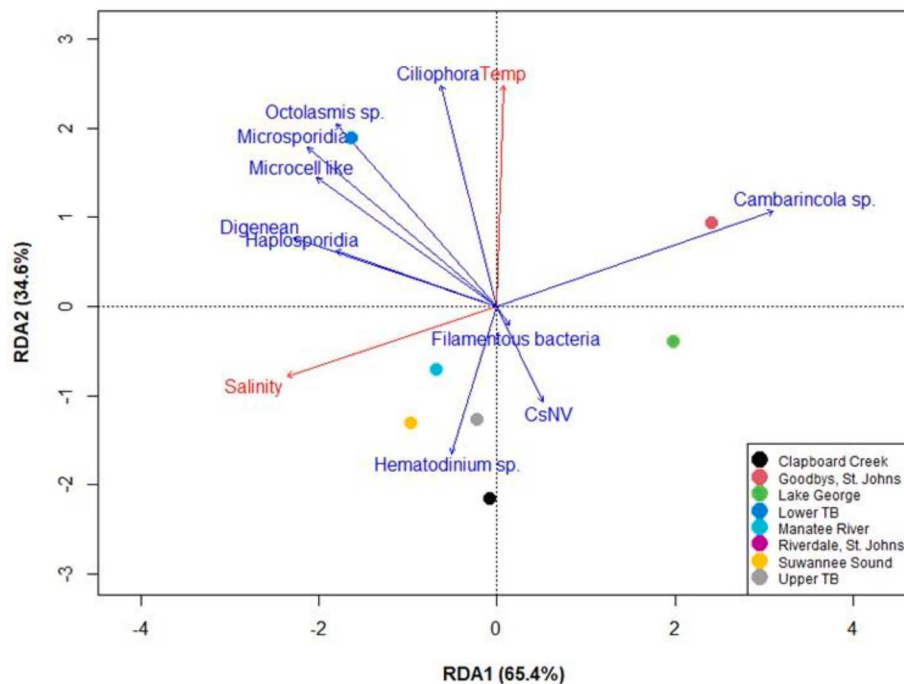


Fig. 9. Ordination diagram of redundancy analysis (RDA) demonstrating the relationship between symbiont taxonomic groups (blue arrows) and the environmental variables (red arrows), salinity, and temperature among the different sampling sites (colored circles). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

unit increase in STR.

Negative binomial regression modeling suggested that two symbiont groups correlated with *C. sapidus* host reflex response, since their 95% confidence intervals did not overlap zero (Table 2). Microsporidian ($n = 19$) had a significant (model estimate = 0.451) effect on host reflex response, where RIS was, on average, 1.57 ($e^{0.451}$) times higher compared to all other symbionts grouped together. Conversely, *Cambarincola* sp. ($n = 72$) had a significant (model estimate = -0.255) effect on host reflex response, where RIS was, on average, 1.29 ($1/e^{-0.255}$)

times lower in comparison to all other symbiont groups (Table 2). The remaining symbiont groups did not significantly correlate with RIS (Table 2). We excluded four symbiont groups (*Hematodinium* sp., gregarines, Nematoda, and CsNV) from analysis because they occurred in < 15 infected animals.

4. Discussion

The majority of Florida *C. sapidus* (77.9%) were found with one or

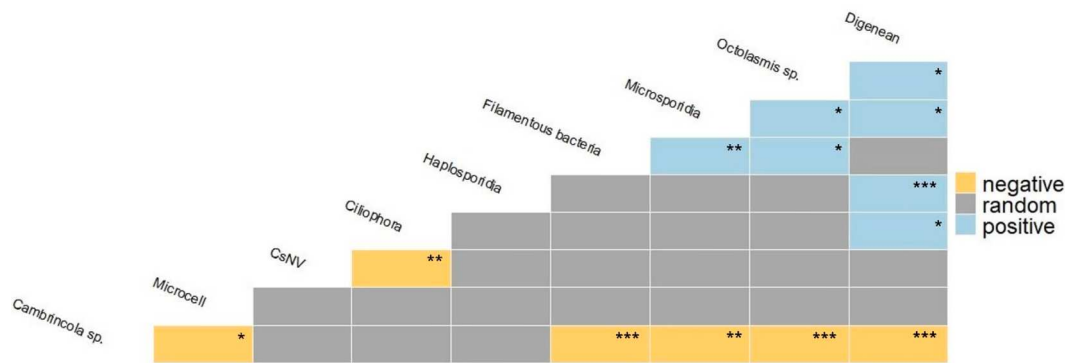


Fig. 10. Co-occurrence relationships among *Callinectes sapidus* symbiont groups. Statistically significant positive (blue) and negative (yellow) relationships among symbiont groups. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 2

AICc, Δ AICc, Akaike weights (*w*), parameter estimates, standard errors (SE), lower and upper 95% confidence limits from the negative binomial regression models relating the presence of symbionts to blue crab RIS scores, and model rate ratios. Parameter estimates are interpreted as the difference between the mean RIS score for blue crabs harboring a given symbiont compared to blue crabs that do not harbor that symbiont. Model intercepts from each model are not reported.

Symbiont	AICc	Δ AICc	<i>w</i>	Estimate	SE	Lower 95% CL	Upper 95% CL	Rate ratios
Microsporidian	1952.2	0	0.373	0.451	0.204	0.051	0.853	1.570
<i>Cambrincola</i> sp.	1926	0.84	0.245	−0.254	0.128	−0.508	−0.006	1.289
<i>Octolasmis</i> sp.	1927.4	2.16	0.127	0.187	0.114	−0.037	0.41	1.206
Microcell-like	1928.3	3.15	0.077	0.286	0.219	−0.145	0.715	1.331
Haplosporidia	1927.1	3.91	0.053	0.235	0.243	−0.246	0.713	1.265
Ciliophora	1929.3	4.1	0.048	−0.075	0.087	−0.245	0.095	1.078
Digenea	1929.6	4.44	0.04	0.082	0.13	−0.173	0.335	1.085
Filamentous bacteria	1929.8	4.65	0.037	−0.059	0.134	−0.323	0.202	1.060

more STR groups and demonstrated a diverse symbiont profile (12 STR groups). We found a significant and positive correlation between RIS and STR score, suggesting symbionts may be important to consider in RAMP research and could increase predictive ability. Crabs infected with microsporidian in particular had RIS scores 1.57 times higher compared to any other symbiont group. Environmental variables temperature and salinity were found influential as they explained 47.9% symbiont variation among different Florida habitats, and mean site salinity was significantly and positively correlated with *C. sapidus* symbiont diversity.

4.1. Effect of parasitism in *C. sapidus*

C. sapidus harbor a diverse assemblage of parasitic, pathogenic, and commensal organisms. *Hematodinium* sp. and microsporidian are well documented due to their deleterious effects and recurring outbreaks that lead to episodic mortality (Shields and Squyers, 2000, Shields and Overstreet, 2007). Proliferation of the microsporidian *Ameson michaelis* is known to negatively alter skeletal muscle tissue protein and burden metabolic processes (Findley et al., 1981), resulting in lethargic behavior (Stentiford and Dunn, 2014). Our results corroborate this negative host association; *C. sapidus* infected with microsporidian were found with RIS that were on average 1.57 times higher compared to any other symbiont group. This result is not surprising as advanced stage infections of microsporidian spores induce remarkable destruction to host skeletal and cardiac myofibers which are progressively replaced by microsporidian spores, causing lethargic and sluggish nature resulting in less reflexivity of the host. No other symbiont recorded in the present study was found with visible densities comparable to those of the microsporidian group. Another significant disease of crustaceans, *Hematodinium perezii*, is known to impose severe physiological cost in advanced infections, producing sluggish behavior and starvation in their host (Taylor et al., 1996, Butler et al., 2014). We were not able to associate *Hematodinium* with increased RIS averages likely because of

low prevalence (*n* = 8). *Hematodinium* infections in Florida are typically highest in the winter months (Gandy et al., 2015). Additionally, the greatest prevalence of *Hematodinium* typically occurs in juvenile *C. sapidus* (Small et al., 2019) and the parasite prefers high-salinity waters (Messick and Shields, 2000). In the present study, sampling occurred in commercially fished traps, where the majority of crabs collected were legal size, and only two winter sampling sites occurred in high salinities (Clapboard Creek and Suwannee Sound). However, given the well documented physiological effects of *Hematodinium*, it is likely this group affects reflexivity, especially in advanced stage infections. Further study and larger sample size would be necessary to confirm the effects on host reflex response. Collectively, we found crab RIS to be positively and significantly related to STR score. The result suggests inclusion of host symbionts may boost predictive ability of the RAMP application for Florida *C. sapidus* and perhaps other parasite burdened crustaceans. It is clear symbiont groups can alter typical behavior through metabolic and physiological means affecting host reflex response during RAMP examination and can contribute variability in RAMP assessments.

Multiple symbiont infections were relatively common in Florida *C. sapidus*, with 38% of crabs presenting two or more infection types (STR groups; Fig. 2). One crab, collected from the Tampa Bay estuary, hosted six different STR groups, demonstrating Florida wild populations do accrue diverse symbiont assemblages. This result is consistent with another portunid crab, *P. pelagicus*, where 4% of wild crabs were infected with six or more parasites (Shields 1992). Euryhaline portunid crabs likely have more diverse symbiont assemblages because they spend time in various habitats with different parasitic communities. Additionally, our model determined that for each 1-unit increase in STR, RIS increases by 1.19 times. This suggests that increased symbiont diversity negatively effects host reflex response, which is particularly important given that 38% of wild caught crabs hosted two or more symbionts. The additive effects of parasitism are broadly unknown, especially in combination with natural stressors, such as water

temperature and salinity. Hosts with multi-parasite loads are likely to suffer cumulative physiological consequences, especially under prolonged and elevated seasonal temperatures (Dove et al. 2005, Shields, 2019) where the added stress of commercial fishing conditions may exceed the typical regulatory capacity of fished organisms (Koolhaas et al. 2011, Stoner, 2012). The acknowledgement of these complex interactions is gaining recognition (Marcogliese and Pietrock 2011), and warrant further study to effectively evaluate how various stressors affect crabs, especially under a warming climate where environmental variables are subject to change.

It is important to note that sub-tropical Atlantic and Gulf of Mexico *C. sapidus* have been detailed with various other symbionts, in which were not documented in the present study. Overall symbiont prevalence documented via histology is largely and likely an underrepresentation since symbiont findings are reported from a small amount of tissue. Thus, it is more likely the case that sub-tropical *C. sapidus* symbiont prevalences are actually greater than what is reported in this research. Other important symbionts and parasites of sub-tropical *C. sapidus* include the Rhizocephalan parasitic castrator, *Loxothylacus texanus*, commonly found in the warm waters of the Gulf of Mexico; specifically in Florida when water temperatures begin to increase in the Spring and Fall months (Hochberg et al. 1992). Sampling efforts in the present study occurred in the summer and winter seasons to capture the highest and lowest water temperatures, which decreased the chance of documenting *L. texanus*. Crabs residing in warm freshwater environments have been documented to host leech species, *Myzobdella lugubris*, and use the posterior margin of the carapace for mating and depositing eggs (Overstreet 1978). Numerous other symbionts and parasites of *C. sapidus* include fouling barnacles such as *Balanus* and *Chelonibia*, pathogenic gray crab disease caused by amoeba *Paramoeba perniciosus*, and bacterial infections from several species of *Vibrio* to name a few (Shields and Overstreet 2007). In general sampling efforts focusing on internal symbionts and parasites primarily documented with histology limit the finding of many other known taxon hosted by *C. sapidus*.

4.2. Florida's environmental influences of *C. Sapidus* infections

Subtropical *C. sapidus* of southern Florida typically endure prolonged and elevated temperatures (>30 °C) in shallow bays, rivers, and marsh systems during the summer months. These elevated temperatures affect ectotherm homeostasis, which increases physiological and metabolic stress, and escalates disease proliferation (Dove et al. 2005). We determined that both water temperature was influential as it and salinity explained 47.9% of *C. sapidus* symbiont variation throughout Florida habitats during the two seasons (winter and summer). This result is not surprising given water temperature is known to drive patterns in symbiont infections seasonally (Huchin-Mian et al. 2018) and salinity limits marine parasite and symbiont distributions (Blakeslee et al. 2021).

Symbiont infections in wild *C. sapidus* vary markedly across its range due to latitudinal temperature differences and variations in their life history strategies (Zhao et al. 2020). This has been demonstrated in reovirus CsRV1, where the greatest prevalence (>20%) of infection was detected at temperate latitudes, and the lowest prevalence (≤10%) documented in the tropics (Zhao et al. 2020). This result could be indicative of infected crabs dying more rapidly at elevated temperatures, as demonstrated in preliminary experimental CsRV1 research (Schott and Bowers unpublished data). Similarly, mortality rates from *Hematodinium* infections have been documented to be ten times higher in *C. sapidus* under elevated temperatures (25–30 °C), compared to infected crabs exposed to mild temperatures (10, 15, and 20 °C), whose survival exceeded 80% (Huchin-Mian et al. 2018). The differing life history strategies and latitudinal differences throughout the *C. sapidus* range are important to understand in terms of 1) host-symbiont distribution patterns among various environmental ranges (i.e., temperature and salinity) and 2) their various effects on wild populations found in different environmental conditions (i.e., freshwater versus saltwater

crabs) within different geographic regions. While *Hematodinium* and CsRV1 are well documented (Huchin-Mian et al. 2018, Zhao et al. 2020), numerous other symbionts documented in our study are lacking information on infection dynamics in relation to its host and environmental variables.

Salinity creates an environmental gradient that influences parasite and symbiont communities in many marine organisms (MacKenzie 1987, Blakeslee et al. 2021). Florida *C. sapidus* symbiont diversity was found strongly influenced by salinity, where increasing salinity corresponded to increased symbiont diversity (STR groups). Comparably, Noga et al. (1994) found bacterial infections *Vibrio* spp. and *Pseudomonas* spp. prevalence to increase in *C. sapidus* occupying more saline habitats. Throughout Florida's expansive coastline (>12,875 km), there are numerous interconnected estuarine bays, salt marshes, rivers, and lakes, all of which are inhabited by *C. sapidus* depending on its life history stage (Perry et al. 1982). This permits a unique dynamic in parasitism, where crabs' year-long active status and movement within various habitats to spawn, mate, or feed exposes them to potentially more pathogens, especially under a warmer climate regime known to enhance parasite proliferation (Huchin-Mian et al. 2018).

Salinity restriction of symbiont distribution likely explains 11 of the 12 co-infection patterns discovered in the co-occur analysis. Five of the six negative co-occurrence associations involved freshwater *Cambarincola* sp. and mid- to high-range salinity groups (i.e., *Octolasmis* sp., microsporidian), where an overlap of these groups is unlikely because of distribution and the symbiont physiology or lifecycle differences. One interesting negative cooccurrence association involves CsNV and Ciliophora. Both groups were ubiquitous in all salinities in the present study, although this result should be interpreted with caution due to the relatively low CsNV occurrence (n = 9). Fouling organisms like ciliates are thought to be shed following molting (Couch 1967, Shields 1992, Kennedy and Cronin 2007); thus crabs noted with ciliates likely have remained in the intermolt stage for some time to allow for recolonization. It is possible that CsNV-infected *C. sapidus* tend to molt less frequently or CsNV colonizes within the intermolt stage, but controlled experiments would be necessary to confirm any relationship. Six of the seven positive symbiont cooccurrence relationships include groups that were common in mid- to high-range salinities (i.e., microsporidian, Haplosporidia, *Octolasmis* sp.) or where overlap is likely within the shared habitat. For instance, it is not surprising that symbiont groups digenean were found significantly associated with Haplosporidia groups such as *Urosporidium crescens*, as they are known to hyperparasitize encysted metacercaria in *C. sapidus* (Couch 1974). All symbionts documented in the present study had water quality (water temperature and salinity) data collected simultaneously, to aid in developing the current knowledge related to their distribution among environmental variables. Filamentous bacteria have previously been described in *C. sapidus* from the Atlantic and Gulf coasts (Johnson 1976, Messick 1998) although the symbionts relationship to salinity has not been defined. In the present study, filamentous bacteria occurred in habitats where salinity exceeded 19 ppt, suggesting their distribution is limited by the dissolved salt content within the water column. The greater diversity of symbionts in elevated salinities also suggests *C. sapidus* populations residing in fresher water may be less susceptible to infection and could be avoiding parasites by seeking lower salinities (MacKenzie 1987, Gandy et al. 2011, Behringer et al. 2018).

The remaining symbiont diversity in near-freshwater habitats (ciliates, Digenea, CsNV, and *Cambarincola* sp.) were groups that appear to cause minimal impairment to their host. This also coincides with anecdotal observations from commercial crabbers that *C. sapidus* pulled from freshwater are "bulletproof" and have better survival odds in comparison to their saltwater conspecifics during the same season. The dominant freshwater ectocommensal, *Cambarincola* sp., does not directly feed on its host, indicating it has minimal effect on the crab (Shields and Overstreet 2007); it was first reported in Maryland *C. sapidus* in 2003 (Gelder and Messick 2006). In the present study, *Cambarincola* sp.

occupied the gill surfaces and the periphery of the gill chamber in nearly all freshwater crabs and were associated with low RIS. Our analyses showed that RIS scores for *Cambarincola* infected crabs were ~ 1.29 times lower than any other symbiont group. This result is consistent with work by Brown et al. (2012), where low to moderate densities of branchiobdellids in crayfish increase host growth, presumably due to effective cleaning and ample epibiont resources (i.e., gill-fouling ciliates) within the host gills, increasing gas exchange and ammonia excretion processes (Brown et al. 2012). This STR group appears to be the only symbiont group in Florida *C. sapidus* limited to freshwater habitats (Holt et al. 2019).

Peritrich and suctorian ciliates are common epibionts that cement themselves to gill lamellae surfaces and do not appear pathogenic. Heavy infestations may interfere with respiration by occluding portions of the lamellae (Couch 1967, Couch and Martin 1982), but this has not been thoroughly examined. These epibionts are thought to be shed during molting, reducing the chance of high infestation (Couch 1967, Kennedy and Cronin 2007). Digeneans may alter host behavior and cause death if intensity of infection is high (Arundell et al. 2019), although this is likely to be rare in wild populations.

Limited information exists for CsNV (*Nudiviridae*) pertaining to its distribution, transmission, and host effects (Bojko et al. 2022a). Nudivirus infections in other crustaceans, such as *Dikerothammarus haemobaphes*, were correlated with increased host activity and increased viral burden (Bojko et al. 2019; Allain et al. 2020). This suggests that the viral infection has the potential to alter host activity and potentially increase typical reflex response or alter metabolic processes; however, the prevalence of CsNV was too low for us to determine if an activity change was altered in *C. sapidus*.

4.3. Conclusions

In the present study, we highlight freshwater *C. sapidus* have fewer symbionts than their marine congeners, and the utilization of a freshwater environment during their lifecycle may promote better population health and improve the condition of crabs in the fishery. Our results demonstrate that symbionts do influence the reflex response in RAMP assessment, suggesting that documenting this previously unaccounted variable will improve predictive capacity of the RAMP application. Additionally, first evaluations of symbiont diversity patterns in Florida *C. sapidus* allow for a refined understanding of symbiont–host dynamics in this economically important species.

The relationship we show between *C. sapidus* symbiont profiles and environmental variables (temperature and salinity) fills crucial knowledge gaps in our understanding of subtropical *C. sapidus* disease ecology. Further work is needed to better understand the ecological relationship between these symbionts and their host, the symbiont profile patterns among all seasons and habitats, and how they differ across *C. sapidus* geographic range. Our results demonstrate a strong link between environmental salinity and host symbiont profiles and diversity, which are important to consider when assessing this economically and ecologically important species and for similar marine decapods.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This research was supported by the state of Florida's commercial licensing and trap tag fees, provided to the FWRI-CRP for blue crab fishery monitoring and research. We would like to acknowledge the commercial fisherman who allowed us on their vessels to collect data and observe fishing practices and for all open-ended discussions. We are

grateful to FWRI/FWCs histology staff for their dedication and care in processing samples and assisting with pathology and symbiont identification. We would also like to thank the FWRI-CRP staff including Berlynn Heres, Samantha Russo, Katharine Becker, Charles Crawford, and John Harrington for their assistance in data collection and sample processing. GDS and KB are funded by the UK Department of the Environment, Food and Rural Affairs (DEFRA) under project FX001. Authors do not declare a conflict of interest.

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