Filtering out parasites: Sand crabs (*Lepidopa benedicti*) are infected by more parasites than sympatric mole crabs (*Emerita benedicti*)

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Two digging decapod crustaceans, the sand crab species *Lepidopa benedicti* and the mole crab species *Emerita benedicti*, both live in the swash zone of fine sand beaches. They were examined for two parasites that infect decapod crustaceans in the region, an unidentified nematode previously shown to infect *L. benedicti*, and cestode tapeworm larvae, *Polypocephalus* sp., previously shown to infect shrimp (*Litopenaeus setiferus*). *Lepidopa benedicti* were almost always infected with both parasite species, while *E. benedicti* were rarely infected with either parasite species. This difference in infection pattern suggests that tapeworms are ingested during sediment feeding in *L. benedicti*, which *E. benedicti* avoid by filter feeding. Larger *L. benedicti* had more *Polypocephalus* sp. larvae. The thoracic ganglia, which make up the largest proportion of neural tissue, contained the largest numbers of *Polypocephalus* sp. larvae. Intensity of *Polypocephalus* sp. infection was not correlated with how long *L. benedicti* remained above sand in behavioural tests, suggesting that *Polypocephalus* sp. do not manipulate the sand crabs in a way that facilitates trophic transmission of the parasite. *L. setiferus* may be a primary host for *Polypocephalus* sp., and *L. benedicti* may be a secondary, auxiliary host.

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ABSTRACT

Two digging decapod crustaceans, the sand crab species *Lepidopa benedicti* and the mole crab species *Emerita benedicti*, both live in the swash zone of fine sand beaches. They were examined for two parasites that infect decapod crustaceans in the region, an unidentified nematode previously shown to infect *L. benedicti*, and cestode tapeworm larvae, *Polypocephalus* sp., previously shown to infect shrimp (*Litopenaeus setiferus*). *Lepidopa benedicti* were almost always infected with both parasite species, while *E. benedicti* were rarely infected with either parasite species. This difference in infection pattern suggests that tapeworms are ingested during sediment feeding in *L. benedicti*, which *E. benedicti* avoid by filter feeding. Larger *L. benedicti* had more *Polypocephalus* sp. larvae. The thoracic ganglia, which make up the largest proportion of neural tissue, contained the largest numbers of *Polypocephalus* sp. larvae. Intensity of *Polypocephalus* sp. infection was not correlated with how long *L. benedicti* remained above sand in behavioural tests, suggesting that *Polypocephalus* sp. do not manipulate the sand crabs in a way that facilitates trophic transmission of the parasite. *L. setiferus* may be a primary host for *Polypocephalus* sp., and *L. benedicti* may be a secondary, auxiliary host.

INTRODUCTION

Parasites can be generalists that infect many host species, or specialists that infect only a small number of host species, or even just one host species. (Poulin 2007; Schmid-Hempel 2011; Loker & Hofkin 2015). A benefit of being a specialist may be increased adaptation to a host species. Specialization should be favoured in

endoparasites that manipulate host behaviour (Adamson & Caira 2011; Fredensborg 2014), because the nervous systems generating behaviour are probably anatomically and physiologically more variable than other types of tissue (Bullock 1993; Bullock 2004; Bullock 2006). Behavioural manipulation often manifests as parasite induced tropic transmission (PITT), in which parasites with multiple host life cycles change the behaviour of one host in such a way as to enhance the likelihood of the host being eaten by a predatory species that is the next host in the parasite's life cycle (Moore 2002; Lafferty & Shaw 2013)

Sand crabs (*Lepidopa benedicti*) and mole crabs (*Emerita benedicti*) are digging anomuran crustaceans in the same superfamily (Hippoidea) (Figure 1), which are both found in the swash zones of sandy beaches in the Gulf of Mexico. Given that they are relatively closely related and found in the same habitat, it is a reasonable hypothesis that they might have similar parasites to each other. *Lepidopa benedicti* is often infected by an unidentified nematode species that does not appear to manipulate host behaviour (Joseph & Faulkes 2014), which might also infect *E. benedicti*.

Other parasites that infect decapod crustaceans where these two species live are larval cestode tapeworms in the genus *Polypocephalus*, which infect white shrimp (*Litopenaeus setiferus*) (Carreon, Faulkes & Fredensborg 2011; Carreon & Faulkes 2014). Although the life cycle of species in this genus is not completely worked out, it seems likely that it has a two part life cycle (Figure 2): crustaceans (Villella, Iversen & Sindermann 1970; Owens 1985; Shields 1992; Hudson & Lester 1994; Brockerhoff & Jones 1995; Payne 2010) and other invertebrates (Cake 1979) for the larval stage, and elasmobranch fishes (e.g., skates and rays) as the definitive hosts for adults (Butler

1987; Call 2007; Koch 2009). There are reasons that could suggest *Polypocephalus* spp. could be either generalists or specialists. On the one hand, *Polypocephalus* spp. larvae infect multiple species from at least two phyla (Cake 1979; Owens 1985; Brockerhoff & Jones 1995), suggesting that species in this genus are generalists. On the other hand, *Polypocephalus* sp. inhabit the nervous system of crustaceans, and appear to manipulate behaviour in *L. setiferus* (Carreon, Faulkes & Fredensborg 2011), which are factors that suggest species in this genus are specialists.

Polypocephalus sp. is also a candidate for studying the manipulation of host behaviour, because the larval stage infects the neural tissue of their decapod crustacean hosts. Being in or near the nervous system would seem to make such manipulation easier for parasites. In white shrimp, increased infection was correlated with increased activity of the host (Carreon, Faulkes & Fredensborg 2011), which was hypothesized to be a case of parasite-induced trophic transmission. A trophically transmitted parasite in a digging crustacean might be expected to change the behaviour of its host so it spends more time above sand (Joseph & Faulkes 2014). *Litopenaeus setiferus* do dig into sand (Eldred et al. 1961; Fuss 1964; Pinn & Ansell 1993), and their increased activity with infection would be consistent with the prediction above.

This paper compares the patterns of infection in *L. benedicti* and *E. benedicti* for both nematode and cestode parasites, and tests whether *Polypocephalus* sp. manipulates the behaviour of *L. benedicti* as they do with shrimp (Carreon, Faulkes & Fredensborg 2011).

METHODS

Sand crabs (*Lepidopa benedicti*) and mole crabs (*Emerita benedicti*) were collected from the beaches of South Padre Island, Texas by turning over sand with a shovel near, and parallel to, the waterline of the shore (Faulkes, in press; Murph & Faulkes 2013). Crabs found in the overturned sand or in the water of the trench created were collected. Individuals were sexed by examining pleopod size (longer in females) and the carapace length was measured with digital calipers. Different individuals were used to study infection by nematodes and *Polypocephalus* sp. To examine infection of nematodes, *E. benedicti* were broken using forceps, and nematodes found in the dissecting dish were counted, following the previous study on *L. benedicti* (Joseph & Faulkes 2014). To examine infection of *Polypocephalus* sp., individuals were anaesthetised by chilling for ~20 minutes on crushed ice, dissected in sea water, and the nerve cord was removed. The nerve cord was cut into smaller sections, which were pinned in dishes lined with Sylgard (Dow Corning). The nerve cords were dehydrated in a progressive ethanol series (70% ethanol for 5 minutes, 90% ethanol for 5 minutes, 100% ethanol for 5 minutes, then 100% ethanol again for 10 minutes), cleared in methyl salicylate on a depression slide, viewed under a compound microscope (Olympus CX41), and photographed (Olympus C-5050 Zoom digital camera), following a previous study of *L. setiferus* (Carreon & Faulkes 2014). In some cases, consecutive images at different focal points in the Z axis were compiled into a single image using Helicon Focus v. 6.7.1 Lite (Helicon Soft Ltd.).

Initial observations of 10 individuals of each species indicated that variation in numbers of parasites infecting *L. benedicti* was sufficient to test whether there was a

correlation between infection and host behaviour. Because few *E. benedicti* were infected, and there was very little variation in the number of parasites of those that were infected, their behaviour was not examined.

Behavioural tests were similar to those described in Joseph & Faulkes (2014). Individuals were video-recorded digging in a tank 300 mm wide × 150 mm deep × 200 mm high, filled with ~75 mm of sand from South Padre Island covered by ~75mm of seawater. Video was recorded with an iPad 3 using Coach My Video v. 4.4 (<http://www.coachmyvideo.mobi>). Individuals were released at the top of the tank, and was filmed until the carapace was covered by sand. The total time was calculated by subtracting the submergence times from release time (rounded down to whole seconds). Individuals made three digging trials, each separated by a 5 minute rest period when the animal was not disturbed to minimize habituation. The average of the three trials was used for analysis.

The behaviour of crabs fell into three basic categories. An individual could (1) immediately dig into sand (“direct”), (2) stay above sand by tailflipping and rowing its legs (Faulkes & Paul 1997) before digging (“swim”), or; (3) remain on the top of the sand, immobile, before digging (“sit”). “Swim” and “sit” are not mutually exclusive. An individual could do both in one trial, in either order. For simplicity of analyses, individuals that both swam and “sat” in their three trials were omitted from analyses that examined individuals.

Descriptive statistical analyses and graphs were made in Origin 2017 (OriginLab Corporation). Nonparametric tests were used for most analyses because of

122 nonhomogenous variation in data distribution. Nonparametric statistical analyses were
123 performed in SPSS v. 23 (IBM).

124 RESULTS

125 The previously reported prevalence of nematodes in *L. benedicti* (87.0%, n = 46)
126 (Joseph & Faulkes 2014) was higher than in *E. benedicti* (0.0%, n = 22) (Figure 3A).
127 Similarly, the prevalence of *Polypocephalus* sp. infection in *Lepidopa benedicti* (98.0%,
128 n = 50) was higher than in *E. benedicti* (18.2%, n = 22) (Figure 3B). The mean intensity
129 of *Polypocephalus* sp. infection (Figures 3B, 4) was significantly greater in *L. benedicti*
130 (range = 1-170, mean = 34.5, SD = 33.0, n = 49; uninfected animals excluded) than *E.*
131 *benedicti* (range = 1-3, mean = 1.5, SD = 1.0, n = 4; uninfected animals excluded).
132 These differences are not because of the overall size of individuals examined: the
133 average size of *L. benedicti* was smaller than *E. benedicti* (Table 1) in both cases.
134 Because there were so few parasites of either species in *E. benedicti*, all further
135 analyses concern only *L. benedicti*.

136 *Polypocephalus* sp. larvae were closely associated specifically with neural tissue,
137 including peripheral nerves to appendages (Figure 4). The larvae often appeared on the
138 surface of ganglia and could sometimes be seen on the dissected nerve cord using a
139 stereomicroscope.

140 There is a significant correlation (Spearman's $\rho = 0.49$, $p = 0.002$, n = 38)
141 between *L. benedicti* size and mean intensity of *Polypocephalus* sp. infection (Figure 5).

142 Like other anomurans, *L. benedicti* have shorter abdomens than familiar
143 decapods like shrimp and crayfish. Because *L. benedicti* are specialized for digging and
144 swimming with thoracic legs 1 through 4, the legs are proportionately more robust.

Thoracic leg 5 is very small and used for grooming. These anatomical features are reflected in the relative sizes of the ganglia in *L. benedicti* compared to other decapod crustaceans. The thoracic ganglia associated with thoracic legs 1-4 are substantially larger than abdominal ganglia 2-6. The fourth and fifth thoracic ganglia and the first abdominal ganglion are fused. The number of larvae in the ganglia differed significantly across the nervous system (Kruskal Wallis = 16.71, df = 6, $p = 0.01$), with thoracic ganglia containing the most larvae, particularly in highly infected individuals (Figure 6).

Contrary to the prediction that more heavily infected animals would spend more time above sand, the mean intensity of *Polypocephalus* sp. infection was not significantly correlated (Spearman's $\rho = -0.233$, $p = 0.16$, $n = 38$) with mean digging time (Figure 7). Size of *L. benedicti* was not significantly correlated (Spearman's $\rho = -0.279$, $p = 0.09$, $n = 38$) with mean digging time (Figure 8), confirming previous findings (Joseph & Faulkes 2014).

The three main behaviours of *L. benedicti* (directly digging into sand, swimming, or remaining stationary, or "sitting") were significantly different (Kruskal-Wallis = 70.76, df = 2, $p < 0.01$) in how long individuals remained above sand (Figure 9). Swimming above sand and remaining stationary on top of it did not differ significantly in the duration of exposure for sand crabs, although "sitting" times had greater variation, resulted in the longest times that sand crabs were exposed.

Individuals showing different behaviour patterns had significantly different mean intensities of infection (Kruskal Wallis = 8.72, df = 2, $p = 0.013$): animals that "sat" at least once had lower infection intensities than those that swam at least once or always dug directly into sand (Figure 10).

DISCUSSION

Two parasite species, an unidentified nematode (Joseph & Faulkes 2014) and *Polypocephalus* sp., infect *Lepidopa benedicti* with much higher prevalence and intensity than in *Emerita benedicti*. In the case of *Polypocephalus* sp., a high prevalence and intensity also occurs in white shrimp (*L. setiferus*) which also dig into sand (Eldred et al. 1961; Fuss 1964; Pinn & Ansell 1993). What distinguishes *E. benedicti* from both *L. benedicti* and *L. setiferus* is the feeding mode. *Emerita* species are filter feeders (Efford 1966), which *L. benedicti* and *L. setiferus* are not. *Lepidopa* species are probably sediment feeders (Boyko 2002). This suggests that ingestion is a common route of *Polypocephalus* sp. infection for *L. benedicti* and *L. setiferus*. Presumably, *E. benedicti* avoid infection because they are filtering food from the water column, which is hypothesized to have extremely low numbers of *Polypocephalus* sp. cysts compared to sand and other surfaces

The lack of parasites in *E. benedicti* in this population is unusual not only because the sympatric *L. benedicti* is infected, but because other populations of *Emerita* species are infected with other parasites (Smith 2007; Oliva et al. 2008; Kolluru et al. 2011; Violante-Gonzalez et al. 2015; Violante-González et al. 2016). Because this study did not run an entire year, however, it is possible that *E. benedicti* infections vary seasonally or spatially, and that this species is heavily infected at other times or places. That this study did not address whether there any substantial variation in infection rates over time means that the differences in nematode infection in *L. benedicti* and *E. benedicti* should be interpreted with cautiously, because the data for *L. benedicti* (Joseph & Faulkes 2014) was collected before the data for *E. benedicti* (this study).

In *L. setiferus*, the greatest number of *Polypocephalus* sp. larvae is in the abdominal ganglia (Carreon, Faulkes & Fredensborg 2011), but in *L. benedicti*, the greatest number is in the thoracic ganglia. This probably reflects which region has the proportionately greater mass of neural tissue available in the two species, although neural mass does not entirely explain distribution patterns across the nervous system (Carreon & Faulkes 2014). Another difference is that in *L. setiferus*, *Polypocephalus* sp. larvae appeared to be more deeply embedded in neural tissue and were rarely visible under a dissecting microscope until the nerve cord was either squashed or cleared. In *L. benedicti*, larvae were in comparatively superficial positions, and could be seen with dissecting microscopes. There also appeared to be less variation in *Polypocephalus* sp. larval size in *L. setiferus* than *L. benedicti* (compare Figure 4 here to Figure 1 in Carreon, Faulkes & Fredensborg 2011).

Polypocephalus sp. does not seem to manipulate *L. benedicti* in a way that would facilitate trophic transmission. Intuitively, one would predict that if *Polypocephalus* sp. were manipulating sand crabs to make them vulnerable to predators, animals with more *Polypocephalus* sp. would be more likely to swim or remain immobile on the top of the sand. In anything, the evidence points towards more heavily infected individuals being more likely to dig into sand immediately. Nevertheless, this result can be viewed as consistent with the results in *L. setiferus*, where higher levels of infection increased activity (Carreon, Faulkes & Fredensborg 2011). Digging directly into sand and swimming could both be considered higher activity by *L. benedicti*.

The apparent difference in parasite-induced behavioural manipulation in *L. setiferus* and *L. benedicti* has several potential explanations. First, the *Polypocephalus*

214 species infecting *L. setiferus* may not be the same species as the one infecting *L.*
 215 *benedicti*. Although both dig in sand, there are differences in the life history of the two
 216 hosts. For example, *L. setiferus* transition from living seagrass beds (Zimmerman &
 217 Minello 1984) to deeper water as they grow, and change preferences for salinity over
 218 their lives (Williams 1984), whereas *L. benedicti* settle into sand after metamorphosing
 219 from a pelagic larva and remain there for their entire lives (Stuck & Truesdale 1986).
 220 These differences in the niches of the host species could be consistent with there being
 221 multiple *Polypocephalus* species. Genetic testing will eventually be able to determine if
 222 there is one cestode species or multiple species. Second, *L. setiferus* may be the
 223 preferred primary host for *Polypocephalus* sp. (perhaps along with other shrimp
 224 species), and *L. benedicti* is a non-preferred auxiliary host. The intensity of
 225 *Polypocephalus* sp. larvae in *L. setiferus* (mean = 97.7, SD = 102.6; maximum 397; n =
 226 53; Carreon, Faulkes & Fredensborg 2011) is approximately triple that of *L. benedicti*
 227 (mean = 34.5, SD = 33.0; maximum 170; n = 49; this study). *Litopenaeus setiferus* may
 228 be more abundant than *L. benedicti*. *Litopenaeus setiferus* is commercially fished, and
 229 annual catches from trawling in the Texas waters of Gulf of Mexico average 7 million
 230 pounds per year (Texas Parks and Wildlife 2002). In contrast, 10 m transects of beach
 231 often yield less than 10 *L. benedicti* individuals (Faulkes, in press; Murph & Faulkes
 232 2013). *L. benedicti* populations have only been sampled in the swash zone (Faulkes, in
 233 press; Faulkes 2014) and its abundance in deeper waters is unknown (it has been
 234 recorded up to 60 m depth; Boyko 2002). Nevertheless, it seems plausible that the
 235 biomass for *L. benedicti*, and thus its potential as host for *Polypocephalus* sp., is lower

236 than *L. setiferus*. Thus, there may be greater selection pressure for *Polypocephalus* sp.
237 to manipulate its primary host but not secondary ones.

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241 LITERATURE CITED

- 242
- 243 Adamson ML, Caira JN. 2011. Evolutionary factors influencing the nature of parasite
244 specificity. *Parasitology* 109: S85-S95.
- 245 Boyko CB. 2002. A worldwide revision of the recent and fossil sand crabs of the
246 Albuneidae Stimpson and Blepharipodidae, new family (Crustacea, Decapoda,
247 Anomura, Hippoidea). *Bulletin of the American Museum of Natural History* 272:
248 1-396.
- 249 Brockerhoff A, Jones MK. 1995. Ultrastructure of the scolex and tentacles of the
250 metacestode of *Polypocephalus* species (Cestoda: Lecanicephalidae) from the
251 blueswimmer crab *Portunus pelagicus*. *International Journal for Parasitology* 25:
252 1077-1088.
- 253 Bullock TH. 1993. How are more complex brains different? One view and an agenda for
254 comparative neurobiology. *Brain, Behavior and Evolution* 41: 88-96.
- 255 Bullock TH. 2004. Neuroethology has pregnant agendas. *Journal of Comparative*
256 *Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology* 185:
257 291-295.

- 258 Bullock TH. 2006. How do brains evolve complexity? An essay. *International Journal of*
259 *Psychophysiology* 60: 106-109.
- 260 Butler S. 1987. Taxonomy of some tetraphyllidean cestodes from elasmobranch fishes.
261 *Australian Journal of Zoology* 35: 343-371.
- 262 Cake EW, Jr. 1979. *Polypocephalus* sp. (Cestoda: Lecanicephalidea): a description of
263 tentaculoplerocercoids from bay scallops of the northeastern Gulf of Mexico.
264 *Proceeding of the Helminthological Society of Washington* 46: 165-170.
- 265 Call G. 2007. A survey of the tapeworm fauna of the cownose ray, *Rhinoptera bonasus*,
266 from the northern Gulf of Mexico with comments on intermediate hosts. Ecology
267 & Evolutionary Biology. University of Kansas: Lawrence. **M.A.**: 141.
268 <http://hdl.handle.net/1808/5536>
- 269 Carreon N, Faulkes Z. 2014. Position of larval tapeworms, *Polypocephalus* sp., in the
270 ganglia of shrimp, *Litopenaeus setiferus*. *Integrative and Comparative Biology*
271 54: 143-148.
- 272 Carreon N, Faulkes Z, Fredensborg BL. 2011. *Polypocephalus* sp. infects the nervous
273 system and increases activity of commercially harvested white shrimp
274 (*Litopenaeus setiferus*). *Journal of Parasitology* 97: 755-759.
- 275 Efford IE. 1966. Feeding in the sand crab, *Emerita analoga* (Stimpson) (Decapoda,
276 Anomura). *Crustaceana* 10: 167-182.
- 277 Eldred B, Ingle RM, Woodburn KD, Hutton RF, Jones H. 1961. Biological observations
278 on the commercial shrimp *Penaeus duorarum* Burkenroad, in Florida waters.
279 *Florida State Board of Conservation Professional Papers Series* 3: 1-139.

- 280 Faulkes Z. The phenology of sand crabs, *Lepidopa benedicti* (Decapoda: Albuneidae).
281 *Journal of Coastal Research*. in press.
- 282 Faulkes Z. 2014. Abundance and size of sand crabs (*Lepidopa benedicti*) on South
283 Padre Island, Texas. In: Z Faulkes, ed.: figshare.
284 <http://dx.doi.org/10.6084/m9.figshare.1057959>. Access date.
- 285 Faulkes Z, Paul DH. 1997. Coordination between the legs and tail during digging and
286 swimming in sand crabs. *Journal of Comparative Physiology A* 180: 161-169.
- 287 Fredensborg BL. 2014. Predictors of host specificity among behavior-manipulating
288 parasites. *Integrative and Comparative Biology* 54: 149-158.
- 289 Fuss CM, Jr. 1964. Observations on burrowing behavior of the pink shrimp, *Penaeus*
290 *duorarum* Burkenroad. *Bulletin of Marine Science, Gulf and Caribbean* 14: 62-73.
- 291 Hudson DA, Lester RJG. 1994. Parasites and symbionts of wild mud crabs *Scylla*
292 *serrata* (Forsk.) of potential significance in aquaculture. *Aquaculture* 120: 183-
293 199.
- 294 Joseph M, Faulkes Z. 2014. Nematodes infect, but do not manipulate digging by, sand
295 crabs, *Lepidopa benedicti*. *Integrative and Comparative Biology* 54: 101-107.
- 296 Koch KR. 2009. The lecanicephalidean fauna of three species of eagle rays of the
297 genus *Aetomylaeus* (Myliobatiformes: Myliobatidae) Department of Ecology and
298 Evolutionary Biology. University of Kansas: Lawrence. **Master's**: 89.
299 <http://search.proquest.com/docview/304910071>
- 300 Kolluru GR, Green ZS, Vredevoe LK, Kuzma MR, Ramadan SN, Zosky MR. 2011.
301 Parasite infection and sand coarseness increase sand crab (*Emerita analoga*)
302 burrowing time. *Behavioural Processes* 88: 184-191.

303 Lafferty KD, Shaw JC. 2013. Comparing mechanisms of host manipulation across host
304 and parasite taxa. *The Journal of Experimental Biology* 216: 56-66.

305 Loker ES, Hofkin BV. 2015. *Parasitology: A Conceptual Approach*. New York: Garland
306 Science.

307 Moore J. 2002. *Parasites and the behavior of animals*. Oxford: Oxford University Press.

308 Murph JH, Faulkes Z. 2013. Abundance and size of sand crabs, *Lepidopa benedicti*
309 (Decapoda: Albuneidae), in South Texas. *The Southwestern Naturalist* 58: 431-
310 434.

311 Oliva ME, Barrios I, Thatje S, Laudien J. 2008. Changes in prevalence and intensity of
312 infection of *Profilicollis altmani* (Perry, 1942) cystacanth (Acanthocephala)
313 parasitizing the mole crab *Emerita analoga* (Stimpson, 1857): an El Niño
314 cascade effect? *Helgoland Marine Research* 62: S57-S62.

315 Owens L. 1985. *Polypocephalus* sp. (Cestoda : Lecanicephalidae) as a biological
316 marker for banana prawns, *Penaeus merguensis* de Man, in the Gulf of
317 Carpentaria. *Australian Journal of Marine and Freshwater Research* 36: 291-299.

318 Payne JT. 2010. Molecular identification of metacestodes infecting bait shrimp in the
319 Gulf of Mexico. Biology. Tennessee Technological University: Cookeville. **M.S.:**
320 60.
321 [http://proquest.umi.com/pqdlink?did=2142187211&Fmt=7&clientId=12926&RQT](http://proquest.umi.com/pqdlink?did=2142187211&Fmt=7&clientId=12926&RQT=309&VName=PQD)
322 [=309&VName=PQD](http://proquest.umi.com/pqdlink?did=2142187211&Fmt=7&clientId=12926&RQT=309&VName=PQD)

323 Pinn EH, Ansell AD. 1993. The effect of particle size on the burying ability of the brown
324 shrimp *Crangon crangon*. *Journal of the Marine Biological Association of the UK*
325 73: 365-377

326 Poulin R. 2007. *Evolutionary ecology of parasites*. Princeton, New Jersey: Princeton
327 University Press.

328 Schmid-Hempel P. 2011. *Evolutionary Parasitology: The Integrated Study of Infections,*
329 *Immunology, Ecology, and Genetics*. Oxford: Oxford University Press.

330 Shields JD. 1992. Parasites and symbionts of the crab *Portunus pelagicus* from
331 Moreton Bay, Eastern Australia. *Journal of Crustacean Biology* 12: 94-100.

332 Smith NF. 2007. Associations between shorebird abundance and parasites in the sand
333 crab, *Emerita analoga*, along the California coast. *Journal of Parasitology* 93:
334 265-273.

335 Stuck KC, Truesdale FM. 1986. Larval and early postlarval development of *Lepidopa*
336 *benedicti* Schmitt, 1935 (Anomura: Albuneidae) reared in the laboratory. *Journal*
337 *of Crustacean Biology* 6: 89-110.

338 Texas Parks and Wildlife. 2002. Executive Summary: The Texas Shrimp Fishery: A
339 report to the Governor and the 77th Legislature of Texas. TPa Wildlife. Texas
340 Parks and Wildlife: Austin, Texas.
341 http://tpwd.texas.gov/publications/pwdpubs/media/pwd_rp_v3400_857.pdf

342 Villella JB, Iversen ES, Sindermann CJ. 1970. Comparison of the parasites of pond-
343 reared and wild pink shrimp (*Penaeus duorarum* Burkenroad) in South Florida.
344 *Transactions of the American Fisheries Society* 99: 789-794.

345 Violante-González J, Monks S, Quiterio-Rendon G, García-Ibáñez S, Larumbe-Morán
346 E, Rojas-Herrera AA. 2016. Life on the beach for a sand crab (*Emerita*
347 *rathbunae*) (Decapoda, Hippidae): parasite-induced mortality of females in

348 populations of the Pacific sand crab caused by *Microphallus nicolli*
 349 (Microphallidae). *Zoosystematics and Evolution* 92: 153-161.

350 Violante-Gonzalez J, Quiterio-Rendon G, Monks S, García-Ibañez S, Pulido-Flores G,
 351 Rojas-Herrera A, Larumbe-Moran E. 2015. Parasite communities of the Pacific
 352 mole crab, *Emerita rathbunae* (Anomura: Hippidae), in sandy beaches from
 353 Guerrero and Michoacán, Mexico. *Open Journal of Marine Science* 5: 468-476.

354 Williams AB. 1984. *Shrimps, lobsters, and crabs of the Atlantic coast of the eastern*
 355 *United States, Maine to Florida*. Washington, DC: Smithsonian Institution Press.

356 Zimmerman RJ, Minello TJ. 1984. Densities of *Penaeus aztecus*, *Penaeus setiferus*,
 357 and other natant macrofauna in a Texas salt marsh. *Estuaries* 7: 421-433.

358

Table 1(on next page)

Size of animals used in study.

1 Table 1. Size of animals used in study.

	<i>Lepidopa benedicti</i>			<i>Emerita benedicti</i>		
Parasite	Mean carapace length	SD	n	Mean carapace length	SD	n
Nematode sp.	11.44 mm	2.83	46	13.44 mm	5.45	22
<i>Polypocephalus</i> sp.	9.95 mm	1.72	49	18.19 mm	3.65	21

2

Figure 1

Digging crab species.

A) *Lepidopa benedicti*. B) *Emerita benedicti*.

Figure 2

Hypothesized life cycle of *Polypocephalus* sp.

Larval stages of cestode tapeworms in the genus *Polypocephalus* infect crustaceans and other invertebrates. These intermediate hosts are presumably ingested by the putative definitive hosts, skates and rays, which are expected to excrete *Polypocephalus* eggs.

Images from the Noun Project (<https://thenounproject.com>): shrimp by Jeffrey Qua, crab by Mallory Hawes, scallop by B Barrett, and skate by Örn Smári Gíslason.

Figure 3

Infection patterns of sand crabs and mole crabs by parasites.

A) Infection of crabs by unidentified nematode species. *Lepidopa benedicti* data redrawn from Joseph & Faulkes (2014). B) Infection pattern of crabs by *Polypocephalus* sp. larvae. Summary statistics: square = mean; line dividing box = median; box = 50% of data; whiskers = 95% of data; triangles = minimum and maximum. Raw data shown by dots.

Figure 4

Micrographs of *Polypocephalus* sp. in nervous tissue.

A) *Lepidopa benedicti* thoracic ganglion 2. B) *Lepidopa benedicti* thoracic ganglia 3, and fused ganglion consisting of thoracic ganglia 4, 5, and abdominal ganglion 1. Different individual than A. C) *Emerita benedicti* brain. Arrow indicates single *Polypocephalus* larvae. D. *Emerita benedicti* thoracic ganglion 1. No *Polypocephalus* sp. larvae in this individual. Different individual than C. Anterior towards top in A, and towards left in B-D.

Figure 5

Bigger *Lepidopa benedicti* have more *Polypocephalus* sp. larvae.

Relationship between size of *L. benedicti* and intensity of *Polypocephalus* sp. infection.

Figure 6

Number of *Polypocephalus* sp. larvae in different regions of the nerve cord in *Lepidopa benedicti*.

Summary statistics: square = mean; line dividing box = median; box = 50% of data; whiskers = 95% of data; triangles = minimum and maximum. Raw data shown by dots. Groups sharing a letter do not differ significantly from each other. Sample sizes vary because some ganglia were damaged during dissection. SEG = subesophageal ganglion; T = thoracic ganglion; A = abdominal ganglion.

Figure 7

Polypocephalus sp. infection does not affect speed of digging in *Lepidopa benedicti*.

Relationship between intensity of *Polypocephalus* sp. infection and digging time in *L. benedicti*.

Figure 8

Size does not affect digging time in *Lepidopa benedicti*.

Relationship between carapace length and mean digging time in *L. benedicti*.

Figure 9

Time above sand for different behaviours by *Lepidopa benedicti*.

Duration of individual behaviour trials, grouped by different behaviours. N = 110 trials. Four trials involved combinations of sitting and swimming, and are not shown due to their rarity.

Summary statistics: square = mean; line dividing box = median; box = 50% of data; whiskers = 95% of data; triangles = minimum and maximum. Raw data shown by dots.

Groups sharing a letter do not differ significantly from each other.

Figure 10

Infection intensity of *Lepidopa benedicti* individuals showing different behaviours.

Individuals categorized into three groups: those that always dug directly; those that swam at least once, but never "sat" (i.e., remaining immobile on the surface); those that "sat" at least once, but never swam. Summary statistics: square = mean; line dividing box = median; box = 50% of data; whiskers = 95% of data; triangles = minimum and maximum. Raw data shown by dots. Groups sharing a letter do not differ significantly from each other.