Missing link: California rock crabs serve as intermediate hosts for the parasite *Helicometrina nimia*

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Abstract

Parasites can have strong effects on invertebrate host behaviour, fecundity and survival in marine ecosystems. However, parasites are often poorly documented and still rarely integrated into marine ecological modelling; comprehensive surveys of infection in marine invertebrates are sporadic at best. For example, rock crabs are an important part of Californian coastal ecosystems, both as regulators of mussel populations and non-native species, and as prey items for predators like sea otters, but their parasite communities and potential effects on crab population dynamics are seldom studied or understood. Here, we present the first report of infection by the trematode *Helicometrina nimia* in the economically and ecologically important red rock crab (*Cancer productus*) and Pacific rock crab (*Romaleon antennarium*). As intermediate hosts, they are a missing link for infection by *H. nimia* in Californian fish that was unreported until now. Based on these findings, we advocate for further research into parasite diversity and their potential effects on ecologically and commercially important species.

Introduction

Digenean trematodes comprise one of the most common parasite groups in marine invertebrates, often infecting multiple host species due to their complex lifecycle stages, and affecting host survival, physiology or behaviour (Mouritsen & Poulin, 2002; Lefèvre *et al.*, 2009; Leiva *et al.*, 2017; Dairain *et al.*, 2019). In recent years, parasites have increasingly been recognized as critical components of marine food webs; they can influence biodiversity and ecosystem functioning (Mouritsen & Poulin, 2005; Lafferty *et al.*, 2008; Frainer *et al.*, 2018; Dairain *et al.*, 2019). The effects of parasitism may be particularly important in marine ecosystems for species that alter the physical structure and flow of nutrients in benthic communities (bioturbators) such as rock crabs (Fanjul *et al.*, 2011; Dairain *et al.*, 2019).

Rock crab commercial fishing has been rapidly expanding in California while research into their population dynamics is limited. Proper management will require accurate ecological information (Fitzgerald *et al.*, 2018). Commercial fishing takes vary between 450 and 900 metric tons of crab annually and is a growing market (CDFW, 2019; Fitzgerald *et al.*, 2019). Recent reports have raised concern about a decline in the population of red (*Cancer productus*), yellow (*Metacarcinus anthonyi*) and brown rock crabs (*Romaleon antennarium*), although these trends could not be attributed specifically to either environmental factors or overfishing (Fitzgerald *et al.*, 2019). Beyond their economic importance, rock crabs also fulfil key ecosystem roles as controllers of non-native species like the European green crab (*Carcinus maenas*) (Jensen *et al.*, 2007) or tunicates (Epelbaum *et al.*, 2009). *Cancer productus* and *R. antennarium* may also be important controllers of mussel populations, which, left unchecked, can become dominant in lower intertidal communities (Hull & Bourdeau, 2017). With the loss of keystone predators due to sea star wasting disease, researchers have suggested that rock crabs may be essential in compensating for sea star population decline (Hull & Bourdeau, 2017). They are also important prey items of the southern sea otter (*Enhydra lutris nereis*) (Fujii *et al.*, 2017), as well as benthic fishes, octopus and bottom-foraging sharks (Carroll & Winn, 1989). Here, we outline the discovery of a digenean parasite in these ecologically and environmentally important species, highlighting a significant gap in knowledge of our understanding of cancrid crab populations in the Eastern North Pacific.

Materials and methods

We surveyed various crab species in central California as part of a broader study investigating the prey of sea otters to discover the intermediate host(s) of their intestinal parasites. Crabs were collected via hand nets and traps in Santa Cruz, Monterey and San Luis Obispo counties from municipal wharfs (Santa Cruz Warf, Monterey Bay Municipal Warf 2, Cayucas Pier and Port San Luis Pier) during the summer of 2019. Specimens were frozen to euthanize, and then
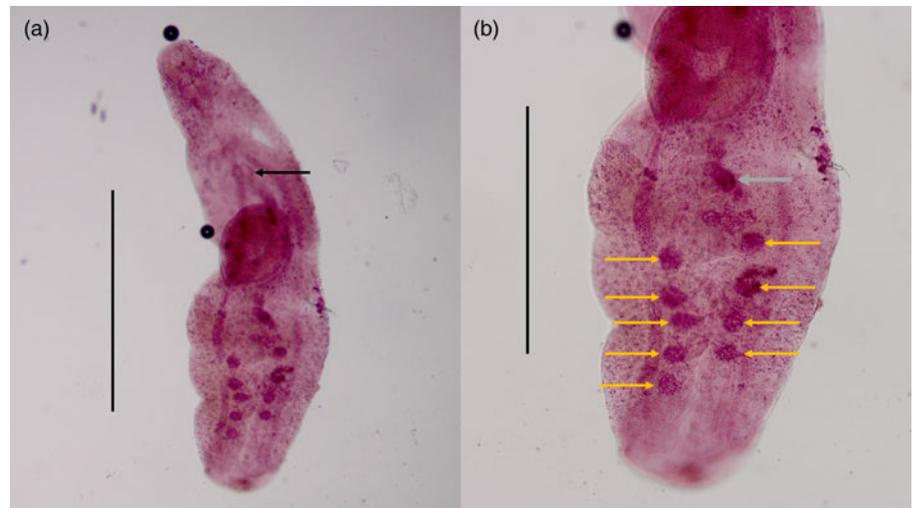
Fig. 1. Metacercaria of *Helicometrina* sp. ex *Cancer productus* stained: (a) whole worm, with the genital pore highlighted (arrow); (b) posterior body showing immature testes (yellow) and ovary (grey). Scale bars: (a) 1 mm; (b) 500 μm.

dissected at the California Department of Fish and Wildlife office in Santa Cruz, California. Species investigated included *Metacarcinus gracilis* ($n = 149$), *Metacarcinus magister* ($n = 4$), *C. productus* ($n = 64$) and *R. antennarium* ($n = 63$). During these investigations, dissections of *C. productus* and *R. antennarium* from the Monterey Bay revealed infections by metacercarial cysts of an unknown trematode. The cysts had a thin brown melanized capsule, were spherical in shape and found within the haemocoel (just inside the carapace and body wall) and joints of the crabs. Metacercariae were manually extracted from their cysts and were examined morphologically and molecularly to determine the species.

For light microscopy, metacercariae were stained with acetic acid carmine, cleared in clove oil and mounted permanently in Canada balsam (fig. 1). Molecular characterization of the 28 s ribosomal RNA (rRNA) large ribosomal subunit gene was conducted with universal primers T16 (5′ GAG ACC GAT AGC GAA ACA AGT AC 3′) and T30 (5′ TGT TAG ACT CCT TGG TCC GTG 3′) (Harper & Saunders, 2001), sequenced via the Sanger method at the University of Otago, New Zealand. Characterization of the 18 s rRNA small ribosomal subunit gene used primers SB3a (5′ GGA GGG CAA GTC TGG TGC 3′) and A27a (CCA TAC AAA TGC CCC CGT CTG) and was sequenced via the Sanger method at the University of Alberta, Canada. Resulting sequences were identified with a nucleotide-sequence BLASTn search via the National Center for Biotechnology Information website (Altschu *et al.*, 1990; Madden, 2002). The 18 s sequences were edited with the software Mega X, and fragments aligned by MUSCLE with the program's default parameters (Kumar *et al.*, 2018). For the two species infected (*C. productus* and *R. antennarium*), we investigated the effect of host size (width of carapace), location of capture, sex and species on infection prevalence (binomial distribution, 'logit' function) and intensity (quasi-Poisson distribution, 'log' function) of the trematode, through Rstudio ($n = 127$) (Rstudio Team, 2015). *Metacarcinus gracilis* was not included in the model as they were not observed to be infected and would have led to zero inflation of the model.

Results and discussion

Morphological examination of the metacercariae identified the specimens as members of the genus *Helicometrina* due to the presence of nine testes and the genital pore located below the caecal bifurcation (fig. 1) (Cribb, 2005). Metacercariae were, on average, 2190 μm long and 590 μm wide (see supplementary material), and the cysts were, on average, 731 μm in diameter. The 28S sequence from metacercariae retrieved from *C. productus* returned a closest match to *Helicometrina nimia* (ex *Haemulon falvolineatum*, French grunt, Mexico) with 97.97% identity (MK648305; see Pérez-Ponce de León & Hernández-Mena, 2019). The 18S sequence from metacercariae retrieved from *C. productus* returned a closest match with *H. nimia*, with 98.93% (KJ995999, ex *Acanthistius pictus*, brick sea bass, Chile; see Gonzalez, 2016). The 18 s sequence from *R. antennarium* also returned a closest match with *H. nimia*, with 98.64% (KY614306, ex *Semicossyphus darwini*, Galapagos sheephead wrasse, Chile; see Ñacari *et al.*, 2018). In three partial 18 s sequences (two ex *C. productus* and one ex *R. antennarium*) with coverage at 307 bp, there were four haplotypes shared by the metacercarial sequences that were different from three exemplar sequences from South America (KJ995995, González *et al.*, 2013; KY614306, Ñacari *et al.*, 2018; KF938641, Oliva *et al.*, 2015). Previous reporting of 18 specimens of *H. nimia* from three different host species found nine variable sites in the 18 s region from a partial sequence of 372 bp, with an average pairwise difference of three (Oliva *et al.*, 2015). We conclude our finding to likely be *H. nimia* based upon comparison to available sequences and morphological similarities. The sequences had some relation to *Helicometrina labrisomi*, but the specimens did not correspond to this species' morphological description (Linton, 1910).

Infection prevalence was 14% in *C. productus* with an average intensity of 11.7 ± 3.2 per infected crab, and 9.5% in *R. antennarium* with an average intensity of 14.3 ± 4.9 parasites per infected crab. Statistical analysis revealed a significant effect of carapace width on prevalence ($P < 0.01$) and intensity ($P < 0.01$), and sex ($P = 0.01$) on intensity, with females having greater intensity of infections (fig. 2). Location of capture, crab species and their interaction had no significant effect in the models and were sequentially removed to create a minimum adequate model. This is interesting as no crabs in San Luis Obispo were infected. The lack of significant effect of location on prevalence may be due to low prevalence and our limited sample size. Future research should expand into broader sampling to determine if infection does occur further south, and if there is a significant difference in infection prevalence.
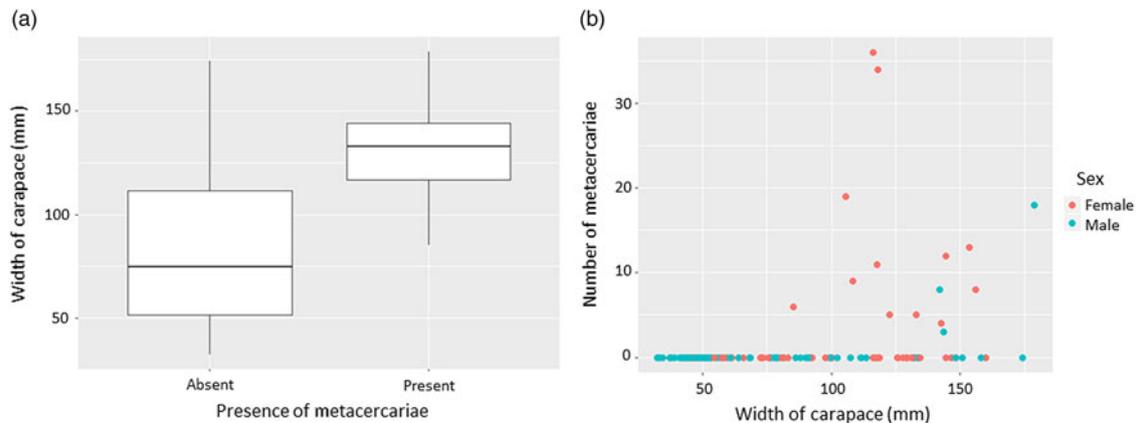
Fig. 2. Effect of carapace width in *Cancer productus* and *Romaleonantennarium* on infection. The two species are pooled as the model showed no significant difference between them. (a) Boxplot of the effect of carapace width on prevalence (presence/absence) of *Helicometrina* sp., median and quartiles. (b) Scatterplot of the interaction of carapace width on intensity of infection (total parasites) by sex of the crab host (male, blue; female, red).

No metacercariae were discovered in any of the Dungeness (*M. magister*) or graceful rock crabs (*M. gracilis*) collected. The lack of infection in *M. gracilis* may be due to differences in habitat and behaviour; *M. gracilis* are much smaller than *C. productus* and *R. antennarium*, and while they co-occur in some habitat, they are also found in shallower, open sandy habitats or eelgrass beds and feed on different prey species (Orensanz & Gallucci, 1988; Orensanz et al., 1995). Since *H. nimia* is not host specific in their decapod hosts in South America (Leiva et al., 2015, 2017), this lack of infection may be due to differences in habitat selection. It should be noted that only four Dungeness crabs were collected, possibly due to the time of year or the difficulty of collection via hand net/trap. Therefore, we cannot comment on whether this species is host for this parasite. Future sampling should target *M. magister* as they are of great economic significance (CDFW, 2019).

Species of *Helicometrina* have previously been reported to utilize various decapod crustaceans as intermediate hosts. In South America, host families include Epialtidae, Porcellanidae and Xanthidae (Leiva et al., 2015, 2017). There exists one report of *Helicometrina* cf. *nimia* infection in a species of Cancridae (*Romaleon polyodon*) in Chile, although this report was from a single crab (Leiva et al., 2015). The definitive hosts of *Helicometrina* spp. are teleost fish. Studies have reported *Helicometrina* spp. in central and South America from fish species in the families Merlucciidae, Serranidae, Pingipedidae, Labrisomidae, Cheilodactylidae, Ophidiinae and Gobiesocidae (Gonzalez et al., 2006; Muñoz & Olmos, 2008; Morales-Serna et al., 2017). In North America, a study found *H. nimia* tended to be a fish generalist (Holmes, 1990). *Helicometrina nimia* has been reported in shiner perch (*Cymatogaster aggregata*) (Arai et al., 1988), and (as *Helicometrina elongata*) in perch (Embiotocidae), Hubbs (Blenniidae) and scorpion-fish (Scorpaenidae) in Southern California (Montgomery, 1957). There is one report of *H. nimia* in Monterey California, where our study was conducted, in various species of fish (Chapa, 1969).

This is the first report of a *Helicometrina* species in crabs of the family Cancridae, from Pacific North America. The last report of this parasite in Californian fish was over 30 years ago (Holmes, 1990). Nonetheless, *H. nimia* is a generalist in terms of its definitive host use (Holmes, 1990; Gonzalez et al., 2006; Muñoz & Olmos, 2008; Morales-Serna et al., 2017) so it is likely that the parasite has remained unreported in the fish fauna until now.

For a first intermediate host, other species of *Helicometrina* use gastropod molluscs (Leiva et al., 2015, 2017). In the Mediterranean, species of Opecoelidae were identified in marine snails and abalone, and the authors noted that the parasites, unlike other trematodes, can infect multiple gastropod intermediate host species (Jousson et al., 1999; Leiva et al., 2017). Other trematodes of the order Plagiorchiida have been seen to use mussels of the family Mytilidae (*Perumytilus purpuratus*) (Muñoz et al., 2012) and scallops (*Argopecten purpuratus*) (Oliva & Sanchez, 2005) as their first intermediate hosts in South America. If *H. nimia* also utilizes mussels as their first intermediate host like their relatives in South America this could be significant, considering the possible importance of crab predation on controlling mussel populations (Hull & Bourdeau, 2017). However, the first intermediate host remains unknown (Leiva et al., 2017). Future research should seek this last missing link in order to complete our knowledge of the life cycle of this parasite in California and gain a better understanding of its potential role and effects in the ecosystem, and on crab populations.

Decapod crabs tend to be prey items to teleost fish early in their development and before they attain a size large enough to avoid predation (except during moulting) (Carroll & Winn, 1989). We would expect that infection then increases in prevalence and intensity with size and age (e.g. over time), due to continued exposure to parasite larvae as well as reduced predation. Our analysis showed a significantly positive relationship between carapace width and both infection prevalence and intensity (fig. 2). Interestingly, infection was not seen in any crab with a carapace width smaller than 85 mm. There are a few possible explanations for this (it may be due to higher rates of predation on smaller crabs as a direct result of infection). Digenean trematodes commonly alter the behaviour of their gastropod intermediate hosts (Mouritsen & Poulin, 2002), and their crustacean intermediate hosts (McCurdy et al., 1999; Hansen & Poulin, 2005; Lagrue et al., 2007; Lefèvre et al., 2009). *Paragonimus* cf. *westermani* alters the behaviour of its decapod host (*Eriocheir japonica*) (Kotsyuba, 2018), *Microphallus turgidus* alters the swimming behaviour of Grass shrimp (*Palaemonetes pugio*) (Gonzalez, 2016) and co-infection by the trematode *Maritrema* sp. and acanthocephalans in the body cavity of shore crabs has been correlated with altered serotonin levels (Poulin et al., 2003). The lack of infection in crabs with a carapace smaller than around 85 mm

may, thus, be indicative of high predation rates in small individuals that are infected. Alternatively, gill physiology and respiration behaviours can affect exposure to trematodes in crabs, which may explain the lack of observed infection in smaller crabs (Smith *et al.*, 2007). Though not all trematodes enter their crab host via the gills, some entering through percutaneous penetration at the leg (Gyoten, 2000). As some of the metacercariae were found in the leg joints of some crabs, percutaneous penetration may be more likely. Differences in habitat selection by juvenile and adults of *C. productus* may also explain the lack of infection in smaller crabs, as adults are more likely to be found in open areas and migrate at night to shallower waters (Orensanz & Gallucci, 1988). The full life cycle of *H. nimia* and how it is transmitted to crabs is unknown. Future investigations of the life cycle and effects of *H. nimia* are essential for our further understanding of the importance of this parasite in marine ecosystems.

Cancer crabs are an important part of marine benthic communities from intertidal to deep water through consumptive and nonconsumptive effects (Fanjul *et al.*, 2011; Boudreau & Worm, 2012; Dairain *et al.*, 2019), as prey items for sea otters and fish species (Carroll & Winn, 1989; Fujii *et al.*, 2017), as secondary controllers of mussel populations (Hull & Bourdeau, 2017) and as non-native species control agents (Jensen *et al.*, 2007; Epelbaum *et al.*, 2009). Due to their increasing socio-economic importance, it is essential that research be conducted to investigate not only their little-known population dynamics but also their parasite communities as well (Fitzgerald *et al.*, 2018, 2019). We suggest specific research investigating the possible behavioural effects of infection, and how this might be affecting the role of cancrid species in Californian marine food webs.

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