

Effects of parasitism on the fecundity of the Pacific mole crab, *Emerita analoga*

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TABLE OF CONTENTS

ACKNOWLEDGEMENT.....	3
EXECUTIVE SUMMARY	3
INTRODUCTION.....	3
MATERIALS AND METHOD.....	5
RESULTS.....	7
Gravid vs. non-gravid	7
Fecundity vs parasitism	7
DISCUSSION	8
APPENDIX	9
LITERATURE CITED	13

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EXECUTIVE SUMMARY

Parasitic infection is a key factor in reduction of reproductive potential for many crustaceans. In this study, we examined the Pacific mole crab, *Emerita analoga*, for their fecundity and infection by the acanthocephalan *Profilicollis altmani*. Parasite intensity was positively associated with host carapace length, but this trend was shown more prominently in gravid females than in non-gravid females. Overall this study suggests that there is no evidence in *P. altmani* affecting the fecundity of *E. analoga*. Additional sampling may alter the results presented here.

INTRODUCTION

Parasites have been shown to impact their hosts reproductive potential in many ways, from castration of the host by destruction of gonads to reductions in fecundity as a result of altering host physiology, to little or no effect (Minchella and Loverde 1981; Thomas et al. 1996; Dezfuli et al. 1999; Merlo et al. 2016). Reduction in fecundity may

greatly affect the overall stability of the crab population. It is not known if parasitism affects the reproductive potential in the Pacific mole crab, *Emerita analoga*.

Emerita analoga, is one of the most abundant filter feeding crustaceans found in the intertidal zone of the Pacific coast (Contreras et al. 1999; Jaramillo et al., 2000). *Emerita analoga* serves as an intermediate host for various endoparasites such as the acanthocephalan *Profilicollis altmani* (Smith 2007). The eggs of *P. altmani* is released into the ocean through the feces of shorebirds such as surf scoter *Melanitta perspicillata* and herring gulls *Larus argentatus* (Reish, 1950). The eggs are ingested by *E. analoga*, through filter feeding, and hatch into acanthella larvae in the host's intestine. The larvae travels to the hemocoel transforming into the resting infective cystacanth stage. The infected host may be eaten by a shorebird, the definitive host, where the adult acanthocephalan reproduces. The eggs are released through the bird's feces in the ocean and consumed by mole crabs, thus completing the parasite's life cycle. *Profilicollis altmani* has been reported to infect 50% of crabs (Bhaduri et al., *in revision*) but it is still unknown if this parasite impacts this crustacean's fecundity. Therefore, the aim of the present study was to (1) compare prevalence and infection intensity of *P. altmani* between gravid and nongravid females of *E. analoga*, (2) compare the average and total parasite size (volume) between gravid and nongravid females, (3) assess the association between parasite intensity and crab egg mass, (4) determine the relationship between crab egg mass and parasite volume, and (5) examine association between parasite intensity and host egg developmental stages.

This research will help me in gaining both field and laboratory experience dealing with parasite-host interactions, which will surely be of benefit as a wildlife biologist with

US Forest Services. Not only will I become more familiar with using various equipment like the dissection scope and digital calipers, but I will also be trained in running various statistical tests to analyze my data. This is a crucial skill required for anyone who will be attempting to have a position beyond a seasonal technician, and lead their own projects for the agency.

MATERIALS AND METHOD

Mole crabs were sampled from the splash zone of Del Monte Beach in Monterey, California (36.80° N, 121.90° W) during September, 2017. Specimens were transported to laboratory and stored in the freezer until further analysis. Freezing ensured the mortality of the crabs but did not cause harm to the cystacanths.

After allowing the crabs to thaw out for at least one hour, the carapace length of each crab was measured using a digital caliper to the nearest 0.01mm. If the individual was gravid, the abdomen with the egg mass was removed and soaked in 10% formalin for ten minutes. The eggs were then separated from the pleopods using a tweezer, and observed under a binocular stereo microscope (Olympus 10X dissecting microscope) to classify it under one of four developmental stages: (1) early stage, where eggs appears bright orange in color and have no patterns (2) intermediate stage, where eggs appear orange-light brown in color and contain black eyespots (3) late stage, where eggs appear brown in color with eye spots and red tick marks (4) larval stage, appears brown-grey in color and is no longer spherical and instead equipped with appendages. These eggs were then filtered under a vacuuming flask for 30 seconds and incubated in 61°C incubator for 5 minutes to get rid of excess fluid. A subsample of 20 eggs were

taken and measured for their diameter to the nearest 0.01mm using a stage micrometer. The entire egg sample was then transferred into a calibrated test tube filled half way with distilled water in order to measure the volume of the eggs through water displacement. These eggs were vacuumed once again and incubated for 24 hours.

All crabs were dissected under the compound microscope to examine their hemocoel and internal organs for the presence of cystacanths. Once found, the cystacanths were transferred into distilled water for 30 minutes to allow for their proboscis to be everted. Once the proboscis was fully everted, the length, measured from the tip of proboscis to the end of its trunk, and the width of each cystacanth was measured using a stage micrometer. After all of the cystacanths have been removed, the crab (including abdomen and pleopods removed from gravid females) was incubated along with the eggs in the incubator for 24 hours. After the 24-hour incubation, the dry weight of the crab and eggs was measured using a digital scale to the nearest milligram.

E. analoga eggs are spherical, and thus we used the average diameter taken from the subsamples to calculate the volume of each egg using the formula $V=4\pi r^3/3$. The total egg count for each gravid crab was calculated by dividing the volume of the total eggs by the average volume of one egg. *P. altmani* cystacanths are close to an ovoid shape, thus, the volume of each cystacanth was calculated using the formula $\pi LW^2/6$, where L and W are length and width respectively (Dezfuli et al. 2001).

RESULTS

Gravid vs. non-gravid

Of the 102 specimens examined, ~25% of them were non-gravid and the remainder of them were gravid; a total of 337 cystacanths were extracted. More gravid crabs were infected than non-gravid crabs. Parasite prevalence, mean intensity, intensity range, mean carapace length, and carapace length range are presented in Table 1. Based on ANOVA test, parasite intensity significantly ($F=12.112$, $R^2=0.161$, $P<0.001$) increases as host carapace length increases in gravid females but not significantly ($F=0.246$, $R^2=0.015$, $P>0.05$) in non-gravid females (Fig. 1). Collectively, parasite intensity and host carapace length have a significant ($F=11.085$, $R^2=0.120$, $P<0.05$) positive correlation. Mean cystacanth intensity in relationship to gravid and non-gravid crabs shows no significant ($P>0.05$) difference. There is no significant ($P>0.05$) difference between the total cystacanth volume of gravid and non-gravid females. There was also no significant ($P>0.05$) difference between the mean cystacanth volume of gravid and non-gravid females (Fig. 2).

Fecundity vs parasitism

The egg count ranged from 73 to 21,220 eggs, and the total egg mass ranged from 2 mg to 151 mg. There was no significant ($R^2<0.001$, $P=0.955$) correlation between total egg mass and parasite intensity (Fig. 3). Of the 65 infected, gravid females, 41 individuals carried stage 1, eight carried stage 2, six carried stage 3, and ten carried stage 4 eggs (Fig. 4). There was no significant difference ($F_3=1.83$, $P>0.05$) between the parasite intensity in each egg stage (Fig. 4). There was also no significant

($R^2=0.006$, $P>0.05$) correlation between total egg mass and mean parasite volume (Fig. 5).

DISCUSSION

This study shows a much higher parasite prevalence (84%) than those reported by Bhaduri et al. (*in revision*) where the prevalence was 50%, such difference can be attributed to the previous study being focused on the entire population, males and females, while this study focused only on females. Because female mole crabs are larger than males, and there is a positive correlation between host size and parasite intensity (Bhaduri et al. *in revision*), it is logical that a study focusing on females only would have higher parasite prevalence than a study that looks at the entire population.

As an ongoing study, our limited data suggest lack of the acanthocephalans effects on the mole crab's fecundity. Similar conclusions have been drawn on New Zealand shore crabs (Latham and Poulin 2002). However, unlike previous studies (Latham and Poulin 2002, Bhaduri et al. *in revision*) there were no drop offs in parasite intensity in the larger crabs. Instead, female *E. analoga* with larger body size is likely to have more parasites than the smaller females. For example, the individual with the most (22) cystacanths had a carapace length of 29.19 mm, which is one of the largest crab that was sampled. Although, there is a positive linear relationship between host body size and parasite intensity, this trend is seen much more prominently in gravid females than in non-gravid females. This may indicate that the non-gravid female mole crabs have a type of defense mechanism against the parasites that is lost or reduced in the gravid females. Caution needs to be taken in making such assumptions because the

sample size is small, especially for non-gravid females, and there is a chance that some of the females we identified as non-gravid may have been recently gravid and released their eggs prior to being captured for the study. However, this is unlikely because mole crabs usually reside in the splash zone only during their reproductive season, and returns further away from the shore once the season is over for those individuals. Additional sampling may change some of the results presented here.

APPENDIX

Table 1. Descriptive statistics for the mole crab *Emerita analoga* showing number of mole crabs (N) infected and uninfected, mean intensity, intensity range, mean host carapace length, and carapace length range. Mean intensity and carapace length is reported as mean \pm 1 SE.

N	Infected (N, %)	Uninfected (N, %)	Mean intensity	Intensity range	Mean carapace length (mm)	Carapace length range (mm)
Gravid (77)	(65, 84.42%)	(12, 15.58%)	4.23 \pm 0.14	1-22	22.86 \pm 3.07	17.56-29.53
Non-gravid (25)	(18, 72.00%)	(7, 28.00%)	2.89 \pm 0.11	1-6	21.41 \pm 4.17	13.34-30.16
Overall (102)	(83, 81.37%)	(19, 18.63%)	3.94 \pm 0.11	1-22	22.59 \pm 3.39	13.34-30.16

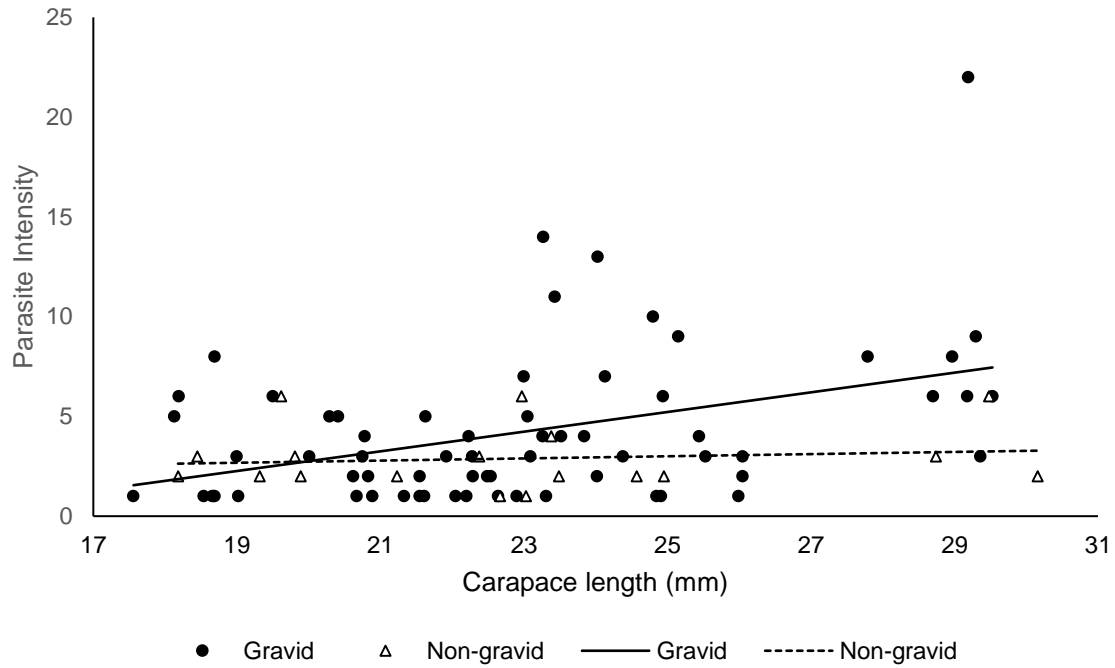


Figure 1: Mean intensity of *P. altmani* cystacanths and host carapace length for gravid and non-gravid *E. analoga*.

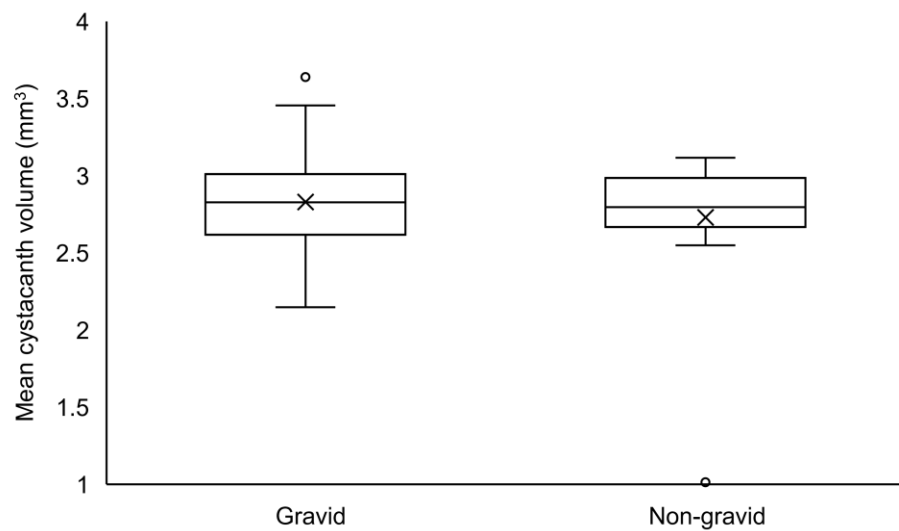


Figure 2: Mean cystacanth volume between gravid and non-gravid crabs.

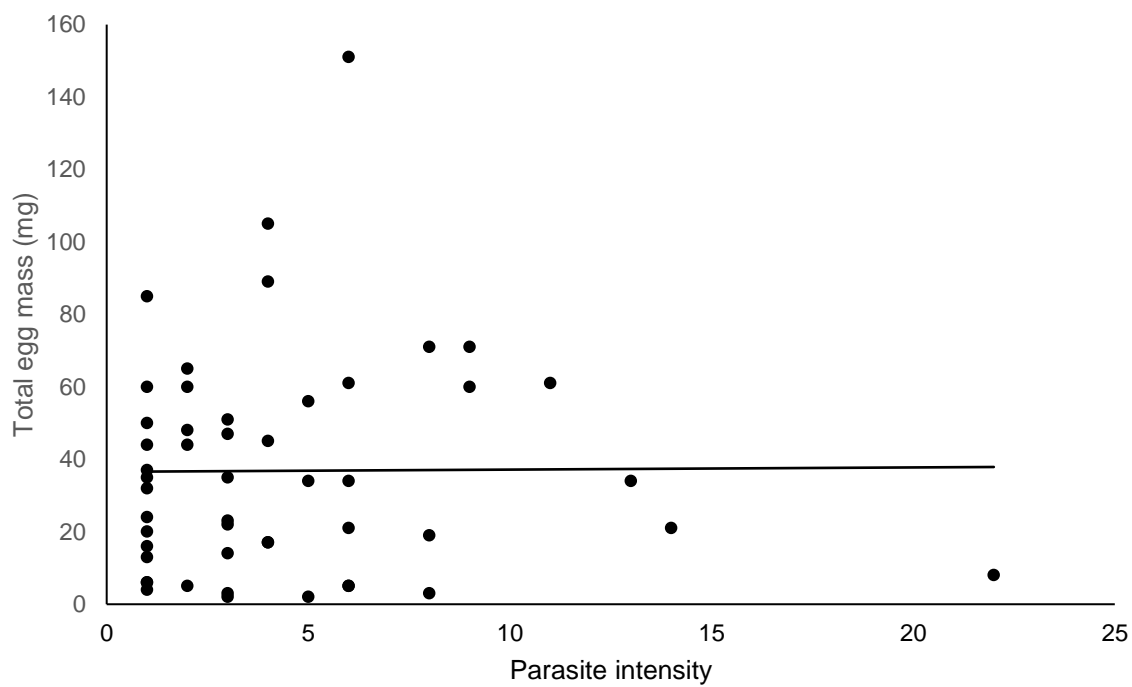


Figure 3: Relationship between total egg mass and parasite intensity.

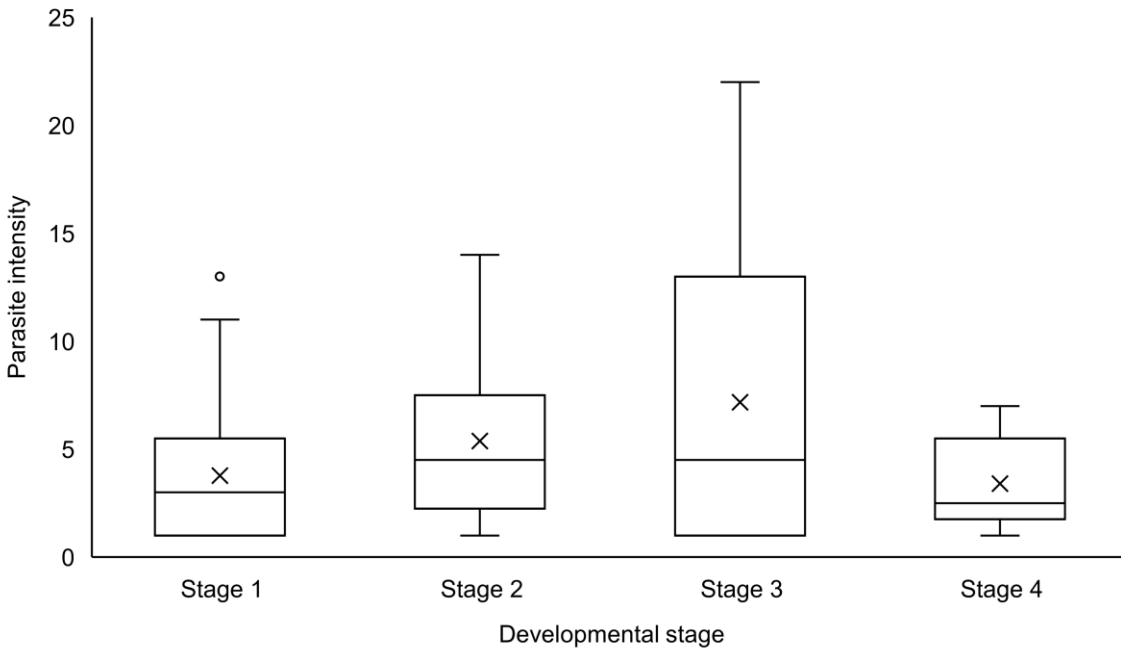


Figure 4: Parasite intensity in relation to egg developmental stage showing mean, median, interquartile ranges, and outliers.

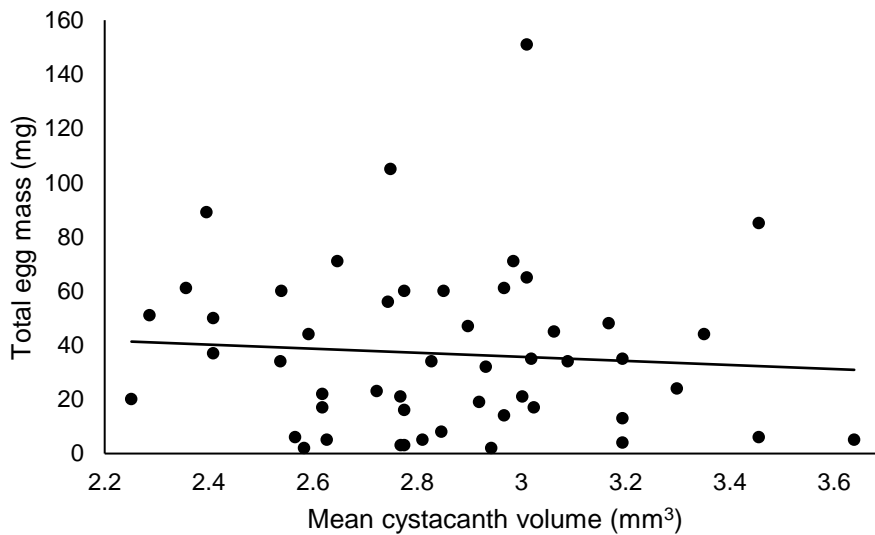


Figure 5: Relationship between total egg mass and mean cystacanth volume.

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