Parasite-induced Behavior Modification to the Circatidal Rhythm of the Atlantic Mole Crab, *Emerita talpoida*

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Abstract

Parasites with complex life cycles require transmission from their intermediate host to their definite host to reach sexual maturity. In some parasite-host systems, parasites manipulate the behavior of their intermediate host to enhance transmission to their definitive host. This mode of transmission is termed parasite increased trophic transmission. While there are many examples of parasites inducing atypical behavior in their hosts, little is known about the ability of parasites to modify host biological rhythms. In this study, I examined the effects of parasite load on the strength of host biological rhythms, using the Atlantic mole crab (*Emerita talpoida*) as a model. Mole crabs are common inhabitants of the swash zone of sandy beaches along the east coast of the United States. They exhibit activity rhythms that are entrained to the tides and act as intermediate hosts for trematode parasites (*Microphallus sp.*) and acanthocephalan parasites (*Profilicollis sp.*). For this study, behavioral assays were performed to quantify the strength of the circatidal rhythms of mole crabs before they were dissected to determine parasite load. On average, rhythmic crabs were found to have significantly greater trematode loads but not acanthocephalan loads compared to arrhythmic crabs. This result is further supported by a logistic regression analysis, which revealed trematode load as the most significant predictor of rhythmicity amongst other demographic variables such as size, sex, ovigerity and month of collection. Overall, results from this experiment support the hypothesis that parasites may influence the biological rhythms of their hosts, presenting an additional mechanism through which parasites may enhance trophic transmission.

Introduction

Modification of host phenotype by parasites once belonged to science fiction stories but is now well described in the field of animal behavior across all major phyla of living organisms (reviewed by Moore 2002). Phenotypic modifications can be divided into two main categories – changes in host appearance and changes in host behavior. A classic example of modification of host phenotype is the infection of terrestrial snails (*Succinea spp.*) by trematodes of the genus *Leucochloridium*. In this parasite-host system, infection results in the alteration of the size and coloration of the snail's tentacles (change in appearance), as well as induces a pulsating behavior in response to light (change in behavior). Together, these changes cause the snail's tentacles to mimic caterpillars, attracting the attention of avian predators, which are the final obligate hosts of the trematode parasites (Moore 2002, Wesolowska and Wesoloski 2014).

The ability of parasites to modulate the phenotype of their host has contributed to idea of the extended phenotype (sensu Dawkins 1982), which views organisms as vehicles through which genes propagate themselves and considers selective pressure at the level of the gene rather than at the level of the organism. In the words of Dawkins (1982): "The phenotypic effect of a gene are the tools by which it levers itself into the next generation, and these tools may 'extend' far outside the body in which the gene sits, even reaching deep into the nervous systems of other organisms." Within the context of parasitism, the expression of a parasite's genes may change the expression of the genes of its host, resulting in an altered phenotype that favors the transmission of the parasite's genes at the expense of host fitness. This notion challenges the traditional position that organisms behave in ways to maximize their own fitness, positing that behavior is not there 'for the good of' the organism, but 'for the good of' genes, and in some cases someone else's genes (Laland 2004).

Parasite-induced host manipulation has been observed across various parasite transmission routes (reviewed by Hughes et al. 2012). In the trophic transmission route, the larval or juvenile stages of a parasite living inside an intermediate host (organism that supports sexually immature parasite stages) must be transmitted to the parasite's definitive host (organism that supports sexually mature parasite stages) by predation for the parasite to complete its life cycle. Host manipulation thus involves the alteration of the appearance or behavior of the intermediate host that leads to increased susceptibility to predation by suitable definitive hosts (Lafferty 1999). Such altered host behavior is thought to be adaptive for the parasites as they facilitate the dispersal or transmission of parasites from their intermediate to definitive host, allowing them to propagate and complete their life cycle (reviewed by Poulin 2010). A clear demonstration of the effect of this phenomenon, termed parasite-increased trophic transmission (Carney 1969, Lafferty and Morris 1996, Thomas and Poulin 1998) can be observed in California killifish (*Fundulus parvipinnis*). Individuals infected with the brain-encysting nematode (*Euhaplorchis californiensis*) were observed to display increased numbers of conspicuous swimming behaviors compared to uninfected individuals, causing them to be up to 30 times more likely to be eaten by birds, the final host of this trematode (Lafferty and Morris 1996, Shaw et al. 2009).

While most recorded host behavioral alterations come in the form of atypical behavior displayed by the host, there are few studies on the modification of host biological rhythms, in other words the frequency and timing of typical host behavior. These studies have largely focused on the effect of Trypanosome infection in small mammals as a model to understand African sleeping sickness, a disease that leads to the fragmentation of the sleep patterns of infected individuals (e.g. Ashman and Seed 1973, Lundkist et al. 2004).

Biological rhythms are observed ubiquitously in nature and influence organismal behavior from the cellular to the whole organism and even population level (Foster and Kreitzman 2005, Tessmar-Raible et al. 2011, Martinez-Bakker and Helm 2015). They function to synchronize an organism's behavior and physiology with natural environmental rhythms, such as day-night cycles (circadian), tidal cycles (circatidal), lunar cycles (circalunar) and seasonal cycles. The rhythmic fluctuations in external or environmental stimuli are termed *Zeitgebers* (German for 'timekeeper') and they entrain an organism's biological oscillator, more commonly referred to as the organism's internal clock (Tessmar-Raible et al. 2011). The general structure of these clocks can be broken down into three components – a sensory input pathway, a central oscillator, and observable rhythmic behavior. The sensory input pathway entrains the central oscillator based on external cues (i.e. *Zeitgebers*), which in turn sustains periodicity and regulates neuronal and hormonal outputs. Changes in these outputs lead to observable rhythmic behavior such as changes in levels of motor activity (Naylor 2010).

Endogenous biological clocks are adaptive and thus evolutionarily advantageous because they allow organisms to synchronize various biological functions with opportune moments in time (Sharma 2003, Martinez-Bakker and Helm 2015). While there are countless examples of the adaptive significance of biological clocks in nature (reviewed in Sharma 2003), a simple illustration is detailed by Kreitzman and Foster (2005): "The eyes of fish take about twenty minutes to change from night-time mode to daylight vision. An animal whose eyes are prepared for the coming of dawn will be able to avoid a predator and catch its prey when the sun rises more efficiently that one who simply reacts to the light." Put simply, endogenous clocks enable organisms to anticipate external changes that are cyclic in nature and adjust their physiology and behavior accordingly.

Of the various types of biological rhythms, circadian rhythms are the most well studied, especially in model species such as *Drosophila melanogaster* (Darlington et al. 1998, Lee et al. 1999, McDonald and Robash 2001) and *Arabidopsis thaliana* (Schaffer et al. 2001). Extensive studies on *D. melanogaster* have revealed several molecular mechanisms of daily rhythms, which operate through feedback loops in the pacemaker genes *period* (*per*) and *timeless* (*tim*). During the day, a transcription factor complex (CLK:CYC) drives *per* and *tim* gene expression through regulatory sequences (E-box elements) in the promoter region while repressing the promoter for *Clk* (Darlington et al. 1998, Lee et al. 1999). However, the double-time protein (DBT) phosphorylates PER, destabilizing it and causing subsequent degradation. Thus, DBT initially prevents the accumulation of PER. Over time, TIM accumulates and interferes with the action of DBT, allowing PER to accumulate around dusk. PER and TIM then dimerize and enter the nucleus in the middle of night, and begin to suppress the function of CLK:CYC. Accordingly, *per* and *tim* translation is halted and an increase in *Clk* translation occurs. At dawn, the photoreceptor CRY is stimulated and proceeds to degrade TIM. The degradation of TIM frees CLK:CYC from the suppressive action of PER and TIM and the clock enters a new cycle (reviewed by Wager-Smith and Kay 2000) This process is the underlying molecular basis that allows an organism to translate the external cycle of day and night to an internal rhythm that controls physiology and behavior.

Apart from circadian rhythms, circatidal rhythms are found widely across marine organisms living in the intertidal zone (summarized in Tessmar-Raible et al. 2011). Intertidal habitats are characterized by the consistent rise and fall in water level, which leads to important consequences for organisms that inhabit them. The changing tides alter abiotic factors such as humidity, salinity, temperature and irradiation as well as biotic factors such as food availability

and exposure to predators (Tessmar-Raible et al. 2011). In response to these constant fluctuations, circatidal rhythms dictate the activity levels of organisms at specific times of the tidal cycle which can influence their distribution across the intertidal zone (Forward et al. 2005). A previous study by our lab (Silliman and Schultz unpublished) aiming to identify the molecular outputs of the circatidal rhythm of the Atlantic mole crab, *Emerita talpoida* (Say), found that many of the tidally regulated genes were more closely related to trematode genes than crustacean genes. This result provides support to the hypothesis of host biological rhythms being potentially modulated by genes from the genome of the parasites they harbor and thus supports Dawkin's (1982) notion of the extended phenotype.

The goal of this study is to explore the effects of parasite infection on the circatidal rhythms of intertidal organisms. Specifically, I examined the influence of parasite load on the strength of the circatidal rhythms of the Atlantic mole crab, *Emerita talpoida*. *E. talpoida* is a species of crustacean found commonly along the Atlantic coast of the United States. They inhabit a region of sandy beaches referred to as the swash zone, which is characterized by a layer of turbulent water that results from the breaking of incoming waves. To deal with the physical disturbance of turbulence from crashing waves and the constant displacement of sand, mole crabs burrow beneath the sand and only ascend into the water column to travel with the flow of water (White 1976). *E. talpoida* is known to exhibit predominantly circatidal rhythms, with activity rhythms peaking just after high tides and minimal activity during low tides (Forward et al. 2005). Even under constant laboratory conditions, *E. talpoida* continues cycling through periods of high activity and inactivity following a semidiurnal rhythm of 12.4 hours, matching the tides from where the crabs were collected (Forward et al. 2005, 2007).

Along the coast of North Carolina, *E. talpoida* acts as an intermediate host for two main groups of parasites – *Profilicollis sp.* (Acanthocephala: Palaeacanthocephala; Nickol et al. 2002), and *Microphallus sp.* (Platyhelminthes: Trematoda; Heard, *personal communication*). Both groups of parasites exhibit complex life cycles that involve intermediate and definitive hosts. The former houses the sexually immature stages of parasites while the latter houses the sexually mature stages. Accordingly, sexual reproduction of parasites can only occur if parasites are transmitted from their intermediate hosts to their definitive hosts, where they become sexually mature.

The phylum Acanthocephala includes parasitic worms commonly known as spiny-headed worms. Their common name is based on the presence of a retractable proboscis (extensible tubular sucking organ) that is covered in sharp hooks, which is a distinguishing characteristic of this group of animals. The life cycle of acanthocephalans begins with the ingestion of freely floating eggs by an appropriate crustacean intermediate host. Once ingested, the eggs develop into a juvenile parasite that encysts in the digestive tract of its host, forming a cystacanth. When the crustacean is then preyed upon by a suitable definitive host, the cystacanth emerges from its cyst, invades the intestine of the final host and develops into an adult worm (refer to Fig. 1).

The ability to alter host phenotype is observed widely across the acanthocephalans and is thought to be an ancestral trait (Moore 1983, 1984). For example, freshwater amphipods (*Gammarus pulex*) infected with acanthocephalans were found to be less photophobic than uninfected individuals, leading to an increased tendency to swim in illuminated areas where they were more vulnerable to predation by fish (Bakker et al. 1997). This modification of host behavior is believed to be adaptive for the parasite by facilitating transmission of the parasite from an intermediate to its definitive host organism.

Trematodes exhibit a similar life cycle to acanthocephalans, with the exception that they typically have a primary and secondary intermediate host. Their life cycle begins with the development of miracidia that hatch from eggs. Free-swimming miracidia infect the primary intermediate host and develop into cercariae. The cercariae then leave the primary intermediate host and infect the secondary intermediate host (typically a crustacean), where they encyst to form metacercariae. When the crustacean is then preyed upon by a suitable definitive host, the metacerciae develop into adult fluke worms (refer to Fig. 2).

Trematodes have been observed to alter the responses of their crustacean hosts to environmental cues, rendering them more susceptible to predation (Helluy and Thomas 2010). In a specific ant-trematode parasitic system, trematodes have also been observed to modify the circadian rhythm of their hosts (Smith Trail 1980). The metacercaria of these trematodes encysts in the central nervous system of the ants, causing them to respond to decreasing temperature and humidity at night by positioning themselves on the top of blades of grass and entering a state of torpor. Thus, the ants are more likely to be ingested by grazing mammals, the definitive hosts of these trematodes.

In the current study, I explored the effects of parasite infection by *Profilicollis sp.* (Phylum Acanthocephala) and *Microphallus sp.* (Class Trematoda) on the circatidal rhythm of *E. talpoida*. I compared parasite loads between individuals showing strong circatidal activity rhythms (rhythmic individuals) and individuals showing weak or no circatidal activity rhythms (arrhythmic individuals) while controlling for size, sex and ovigerity. Circatidal activity rhythms were recorded by placing mole crabs from the wild in individual acrylic columns and recording the number of vertical ascents made for every half hour interval over multiple tidal cycles. Algorithms meant for biological rhythm studies (Deckard et al., 2013) were then used to detect

periodic signals in the activity rhythm data and to designate individuals as rhythmic versus arrhythmic. Upon completion of the activity rhythms, the mole crabs were dissected and the endoparasites present were identified and counted.

Based on the results of the transcriptomic analysis conducted previously by the lab (Silliman and Schultz, unpublished), where a large proportion of tidally regulated genes in *E. talpoida* appeared to originate from trematode parasites, I expected rhythmic individuals to have higher parasite loads and arrhythmic individuals to have lower parasite loads. Additionally, I expected that any effects of parasite load on the circatidal rhythm of *E. talpoida* serves to make them more susceptible to predation by the secondary hosts of the parasites they harbor (e.g. shore birds such as Willets (*Tringa semipalmata*), Sanderlings (*Calidris alba*) and Gulls (Family Laridae)). For instance, parasites may induce peak activity in *E. talpoida* when their avian predators are most actively foraging, making them more susceptive to predation. This hypothesis will be tested by the lab in subsequent field experiments. Together, confirmation of these hypotheses would provide evidence for an alternative mechanism through which parasites can influence trophic interactions within an intertidal community and bring us one step closer towards a more complete understanding of the influence of parasites in structuring communities.

Materials and Methods

Collection

Emerita talpoida were collected during the day in the swash zone at Atlantic Beach Pier, North Carolina, USA, from May to July 2016. *E. talpoida* demonstrates a size-distribution in the swash zone, with individuals decreasing in size in a landward direction (White 1976). Collection was carried out both at high tide and at low tide to capture a wide size-distribution of individuals.

Carapace length (CL) and ovigerity (whether females bore eggs or not) of the crabs were recorded and individuals were sexed based on the presence of specialized pleopods used for carrying egg masses. Crabs were held in seawater with approximate salinity of 35 parts per thousand (ppt) under room fluorescent lights (cool white, 1.1 x 10^{15} photons cm^{-2} s^{-1}) until used in activity rhythm assays.

Activity Essay

The circatidal activity rhythms of *E. talpoida* were characterized by quantifying the frequency of vertical swimming activity. Crabs were placed individually in rectangular Plexiglass columns (38 x 55 x 250 mm) having about 30 mm of autoclaved sand from the collection site on the bottom and 35 ppt seawater. Upon placement into the plexiglass columns, crabs descended and burrowed completely into the layer of sand. A previous study by Forward et al. (2005) found no effects of fluorescent light on mole crab rhythmic activity. Therefore, our activity assays were conducted under constant fluorescent light. Activity assays began four hours before the evening tide on the day of collection and crabs were held under constant conditions of temperature (26^{o}C), light and gentle bubbling aeration.

Activity rhythms of the crabs were recorded continuously at 1 frame per second for 24 or 48 hours using a digital video system (LOREX MPX 1080P Weatherproof Night Vision Security Cameras). For the first seven trials of assays, motion was recorded for 24 hours (two tidal cycles). Recording time was extended to 48 hours (four tidal cycles) for the subsequent 10 trials. The extension from 24 hours to 48 hours was done to capture more tidal cycles and thus to provide a more robust data set to be analyzed by the biological rhythm algorithms. While in the columns, crabs predominantly remained burrowed in the sand and exhibited bouts of activity by

emerging from the sand and swimming towards the surface of the water. Behavior was quantified by counting the number of ascents in each 30-minute interval over the course of the assay. An ascent was counted if the entire crab cleared the sand surface. To expedite the counting process, the video output from the digital video system was converted into jpeg images using FFmpeg (http://www.ffmpeg.org). The resulting time-lapse sequence was imported into ImageJ (Schindelin et al. 2015) and processed using the re-slice and Z-project tools. The output from ImageJ was a composite image detailing the vertical movement of a crab over a 30-minute interval. Counts were performed using the composite image outputs and a time series was constructed for each individual.

Analysis of Activity Assay

To detect periodic signals in the activity assay, two different algorithms were applied to the dataset – Lomb-Scargle (LS) and JTK_CYCLE (JTK). Both algorithms were deemed effective for detecting periodic signals in biological data (Deckard et al. 2013). The LS method (Lomb 1976, Scargle 1982) was conceived in the field of astrophysics and measures correspondence to sinusoidal curves (Glynn et al. 2006). The JTK method originated in statistics but was eventually adapted for biological data (Hughes et al. 2010). It correlates pairs of points and measures correspondence to a reference curve. Given that the goal of this analysis was to detect circatidal rhythms, both algorithms were set to detect rhythmic activity with a period of 12 ± 4 hours. The outputs for both algorithms were *P*-values that corresponded to the likelihood of rhythmic activity for each time series. Time series that reported a P-value of less than 0.05 for either LS or JTK were designated as rhythmic, while the remainder were designated as arrhythmic.

Quantifying Endoparasites

The identity and load of the endoparasites present in the mole crabs was determined by dissecting each crab and observing the contents of its digestive tract under a dissecting scope. The cystocanths of *Profilicollis sp.* and the metacercariae of *Microphallus sp.* were visually identified, counted and recorded.

Statistical Analysis

To determine if parasite loads affect the strength of the circatidal rhythm of mole crabs, the mean load of *Profilicollis sp.*and *Microphallus sp.* for rhythmic versus arrhythmic individuals was compared using a *t*-test. Although the distribution of both *Profilicollis sp.*and *Microphallus sp.* load are right skewed, a large sample size (n = 105 for arrhythmic crabs and n = 131 for rhythmic crabs) allowed for the use of parametric tests. Additionally, to take into the account the effects of other demographic and experimental parameters, a logistic regression analysis was then performed. *Profilicollis sp.* load (N_{acan}), *Microphallus sp.* load (N_{trem}), carapace length (CL), sex, ovigerity, and month of collection were set as predictor variables of the binary output of rhythmicity versus arrhythmicity. Given that the distributions of N_{acan} and N_{trem} were skewed to larger values, a log transformation was applied on these variables before proceeding with the logistic regression analysis. Statistical analysis was performed on JMP Pro 12 (SAS Institute 2015).

Results

Demographics

A total of 236 crabs were collected and analyzed. The size distribution was unimodal and slightly skewed to the right, with half of the crabs having a carapace length between 24.4 mm to 29.5mm (Shapiro-Wilk W test, $W = 0.953$, $P < 0.01$; Fig. 3). Given that sexual dimorphism occurs in *E. talpoidea* (Forward et al. 2005), where females (~25mm) tend to be larger than males (~12mm), a skewed sex ratio was observed with 94 percent of the crabs being female and 6 percent being male. 61 percent of the females were gravid.

Parasite loads for *Microphallus sp.* occurred in greater numbers with metacercariae numbering in the hundreds whereas fewer than ten cystocanths of *Profilicollis sp.* were encountered in dissected samples. The distribution of both *Microphallus sp.* load and *Profilicollis sp.* load were right skewed, with 61 percent of crabs having a *Microphallus sp.* load of less than 100 metacercariaes and 71 percent of crabs being uninfected by *Profilicollis sp.* (Fig. 4).

The rhythmicity assignment based on the Lomb-Scargle and JTK_CYCLE algorithms produced an even distribution, with 131 individuals assigned as Rhythmic and 105 individuals assigned as Arrhythmic. Out of the 131 individuals assigned as Rhythmic, 112 were deemed rhythmic by both algorithms, while the remainder were only deemed rhythmic by either algorithm (13 for LS and 6 for JTK). Overall, there was a consistent agreement between the assignments by LS and by JTK with 91.9% of sampled crabs obtaining the same assignment by both algorithms. Selected time series and their corresponding *P*-values from the JTK and LS algorithms are presented in Figure 5 to provide a better picture of what constitutes a strong rhythmic signature for the JTK and LS algorithms.

Parasite load comparison of rhythmic and arrhythmic crabs

A comparison of the mean parasite load between rhythmic and arrhythmic crabs revealed that *Microphallus sp.* load was significantly greater in Rhythmic crabs (Student's *t*-test, df = 219.3, *t*-ratio = 1.715, $P = 0.044$; Fig. 6), whereas *Profilicollis sp.* load was not significantly different (Student's *t*-test, $df = 215.2$, *t*-ratio = 0.513, $P = 0.304$; Fig. 7).

Regression Analysis

In testing whether parasite load and other demographic characteristics were significant predictors of rhythmicity in *E. talpoida*, the β coefficients and their corresponding P-values based on χ^2 tests of independence from the logistic regression were analyzed. Table 1 summarizes the results from the logistic regression analysis. Of the 6 predictor variables, only log-transformed trematode load was marginally significant ($\beta = -0.230$, $\chi^2 = 3.737$, $P = 0.053$). The remaining predictor variables, which corresponded to acanthocephalan load, month of collection, size, sex and ovigerity had P-values greater than 0.1.

Discussion

Parasite-increased trophic transmission is a well-established phenomenon in parasite ecology. Previous studies have demonstrated that parasite-induced modification to host appearance and behavior may facilitate parasite transmission by increasing the susceptibility of predation of the intermediate host by definitive hosts (Bakker et al. 1997, Lafferty and Morris 1996). The motivation for this study was to investigate if parasite transmission could be facilitated by an additional mechanism – the modification of host biological rhythm. Accordingly, the main goal of this study was to determine the influence of parasite load on the

strength of the circatidal rhythms of the Atlantic mole crab, *E. talpoida*. To this end, results from this study reveal two important insights.

First, although invertebrate inhabitants of intertidal habitats are known to have well-developed tidal rhythms (reviewed in Tessmar-Raible et al. 2011), this study demonstrated that the strength of circatidal rhythms varies significantly from individual to individual. Out of the 236 mole crabs analyzed, only 55 percent displayed a strong rhythmic output. Rhythmic individuals displayed peak activity 1-2 h after high tide during the ebb tide (Fig. 5a, b) while arrhythmic individuals displayed activity levels that remained low for the entire duration of the assay or varied in an erratic manner (Fig. 5g, h). This is consistent with observations by Forward et al. (2005).

Second, comparisons between the mean parasite loads of rhythmic versus arrhythmic individuals suggests that there is a relationship between parasite load and strength of circatidal rhythms in *E. talpoida*. Based on the observation by Silliman and Schultz (unpublished) that a large proportion of tidally-regulated genes in *E. talpoida* corresponds to the trematode genome, I hypothesized that parasite loads would be greater in rhythmic individuals than in arrhythmic individuals. Results from this study are partially consistent with my hypothesis and show that, on average, rhythmic individuals had significantly higher *Microphallus sp.* (Trematode) loads (Fig. 6) but not *Profilicollis sp.* (Acanthocephalan) loads (Fig. 7). This trend remains the same even after demographic effects are factored in using a logistic regression analysis (Table 1). Given that both trematodes and acanthocephalans are notorious for altering the behavior of their crustacean immediate hosts (reviewed in Moore 2002), it is interesting that only *Microphallus sp.* loads were significantly higher in rhythmic crabs. Given this scenario, if both parasites are

destined for the same host species, *Profilicollis sp.* may be "hitching a ride" and taking advantage of the behavioral modifications induced by *Microphallus sp.*.

While the results lend support to our hypothesis of a relationship between parasite load and strength of circatidal rhythms, some caveats should be noted. For instance, the sex ratio of mole crabs sampled is highly skewed, with 94 percent of sampled crabs being female. Given that both endoparasites are housed in the digestive tract of *E. talpoida*, I expected size of the crab to have a strong influence on parasite load. Essentially, the larger a mole crab, the more space there would be in its digestive tract to accommodate endoparasites. To account for this effect, the sampling of mole crabs for this experiment was designed to give us an even size distribution (Fig. 3). However, given that sexual dimorphism is present in *E. talpoida*, where females are significantly larger than males, a concomitant effect was a skewed sex distribution. Accordingly, if sex-specific differences in circatidal rhythms do occur, our sample does not take them into account effectively. The study by Forward et al. (2005) did not detect significant differences in the circatidal rhythms between male and female mole crabs and thus I expect this difference to be negligible.

The circatidal rhythms of intertidal organisms determine their distribution on sandy beaches along a landward-seaward axis. Organisms actively swimming in the water column during the rising tide are carried in the landward direction whereas those active during the ebb tide are carried in the seaward direction. Activity assays performed in this study show that mole crabs tend to be inactive during the rising tide and active during the onset of the ebb tide. As suggested by Forward et al. (2005), this pattern is presumably an adaptation to minimize landward transport and consequently the likelihood of being stranding when the tide recedes. Having strong circatidal rhythms is thus beneficial for mole crabs as it prevents them from

suffering from desiccation or predation due to beach stranding. Accordingly, given the benefits of a strong circatidal rhythm, it seems counter intuitive that rhythmic individuals had higher parasite loads than their arrhythmic counterparts when one might expect otherwise.

Within the context of parasite increased trophic transmission, a possible explanation for this counter intuitive observation is that strong host circatidal rhythms are also adaptive for trematode parasites and thus trematodes may manipulate their host in a way that strengthens host circatidal rhythms. For instance, if the peak activity of mole crabs (as driven by their circatidal rhythms) coincides with periods where the definitively hosts of trematode parasites are actively foraging, a strong circatidal rhythm in mole crabs would be beneficial for the trematode parasites due to increased susceptibility of predation and consequently transmission.

For parasite manipulation to be considered adaptive, it should increase the transmission rate of the parasite and shows some level of specificity in the intermediate host's susceptibility of predation by the definitive host (Cézilly and Perrot-Minnot 2005, Varaldi et al. 2006). As suggested by Lagrue et al. (2007) a useful field approach to determine if parasite manipulation is adaptive is to compare the stomach contents of definitive host predators with available prey for prevalence of the manipulative parasites. Future studies could employ this approach to determine if strong circatidal rhythms in mole crabs are indeed adaptive for the parasites they harbor.

Although this study provides evidence of a relationship between parasite load and the strength of host circatidal rhythms, it does not determine if parasites are the drivers underlying the observed trends. To determine causality, experimental infections should be carried out. Uninfected *E. talpoida* reared in the lab can be differentially exposed to *Microphallus sp.* and *Profilicollis sp.*, and their subsequent behavior can be monitored and quantified to detect differences between infected and uninfected individuals.

Finally, for a more comprehensive understanding on how parasites may modify the biological rhythms of intermediate crustacean hosts, possible physiological mechanisms through which this interaction can occur must be explored and identified. This may prove a challenge given our limited understanding of the molecular machinery underlying circatidal rhythms. While circadian pacemakers have been identified within discrete neuroanatomical sites in terrestrial model organisms (e.g. the suprachiasmatic nucleus in mammals – Klein et al., 1991; lateral neurons in *Drosophila* – Helfrich-Forster et al. 2007), the same cannot be said for circatidal pacemakers. Specific to decapod crustaceans, recent studies point to the supraesophageal ganglion (brain), the optic ganglia and the X-organ-sinus gland (XO-SG) complex as putative sites for biological clocks (Horacio et al. 2010). For the crab *Carcinus maenas*, removal of the eyestalks resulted in arrhythmic locomotor activity, suggesting that the XO-SG complex is the site of the circatidal clock (Naylor 1968). Thus, to show conclusively that the trematode parasite *Microphallus sp.* is indeed directly influencing the strength of circatidal rhythms in *E. talpoida*, future studies would have to investigate possible means through which *Microphallus sp.* metacercariae, which is housed in the gut, is able to modulate activity in the XO-SG complex of *E. talpoida*. This is likely to involve the secretion of substances that interact with host neurochemistry (Thomas et al. 2005). For example, the larval stages of trematodes have been documented to induce changes in the concentrations of neurotransmitters in crustacean hosts (Helluy and Thomas 2010).

In summary, this study suggests the possibility of parasites actively manipulating host rhythms to enhance trophic transmission. While the results show a significant relationship between trematode parasite load and strength of circatidal rhythms in mole crabs, more studies are needed to conclusively pinpoint the former as the proximate cause of the latter. At the

ecological level, by altering the predation rate of infected intermediate hosts, manipulative parasites may serve as potential regulatory factors of the density of prey populations. Thus, determining if modified host biological rhythms are a possible mechanism through which manipulative parasites operate is important in developing a more holistic understanding of parasite induced trophic transmission and its concomitant role in community ecology.

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References

Ashman, P. U., and J. R. Seed. 1973. Biochemical studies in the vole, Microtus montanus—II. The effects of a Trypanosoma brucei gambiense infection on the diurnal variation of hepatic glucose-6-phosphatase and liver glycogen. Comparative Biochemistry and Physiology Part B: Comparative Biochemistry 45(2):379-392.

Bakker, T., Mazzi, D., and S. Zala. 1997. Parasite-induced changes in behavior and color make Gammarus pulex more prone to fish predation. Ecology 78(4):1098-1104

Carney, W. P. 1969. Behavioral and morphological changes in carpenter ants harboring dicrocoeliid metacercariae. The American Midland Naturalist 82(2):605-611.

Cézilly, F., and M. J. Perrot-Minnot. 2005. Studying adaptive changes in the behaviour of infected hosts: a long and winding road. Behavioural processes 68(3):223-228.

Darlington, T. K., Wager-Smith, K., Ceriani, M. F., Staknis, D., Gekakis, N., Steeves, T. D. L., Weitz, C. J., Takahashi, J. S., and S. A. Kay. 1998. Closing the circadian loop: CLOCK-induced transcription of its own inhibitors per and tim. Science 280:1599-1603.

Dawkins, R. 1982. The extended phenotype: The gene as the unit of selection. Oxford University Press, Oxford, United Kingdom.

Deckard, A., Anafi, R. C., Hogenesch, J. B., Haase, S. B., and J. Harer. 2013. Design and analysis of large-scale biological rhythm studies: a comparison of algorithms for detecting periodic signals in biological data. Bioinformatics 29(24):3174-3180.

Friend, M., and J. C. Franson. 1999. Field manual of wildlife diseases: general field procedures and diseases of birds. Information and Technology Report. Washington, D.C. Retrieved from http://pubs.er.usgs.gov/publication/itr19990001

Forward, R. B., Diaz, H., and J. H. Cohen. 2005. The tidal rhythm in activity of the mole crab Emerita talpoida. Journal of the Marine Biological Association of the United Kingdom 85(04):895-901.

Forward, R. B., Thaler, A. D., and R. Singer. 2007. Entrainment of the activity rhythm of the mole crab Emerita talpoida. Journal of experimental marine biology and ecology 341(1):10-15.

Glynn, E. F., Chen, J., and A. R. Mushegian. 2006. Detecting periodic patterns in unevenly spaced gene expression time series using Lomb–Scargle periodograms. Bioinformatics 22(3): 310-316.

Helfrich-Förster, C., Yoshii, T., Wülbeck, C., Grieshaber, E., Rieger, D., Bachleitner, W., Cusumano, P., and F. Rouyer. 2007. The lateral and dorsal neurons of Drosophila melanogaster: new insights about their morphology and function. Cold Spring Harbor symposia on quantitative biology 72:517-525.

Helluy, S. and F. Thomas. 2010. Parasitic manipulation and neuroinflammation: Evidence from the system Microphallus papillorobustus (Trematoda)-Gammarus (Crustacea). Parasites & vectors 3(1):1.

Horacio, O., and Y. W. A. Hsu. 2010. Biological clocks and rhythms in intertidal crustaceans. Frontiers in Bioscience 2:1394-1404.

Hughes, D. P., Brodeur, J., and F. Thomas. 2012. Host manipulation by parasites. Oxford University Press, Oxford, United Kingdom.

Hughes, M. E., Hogenesch, J. B., and K. Kornacker. 2010. JTK_CYCLE: an efficient nonparametric algorithm for detecting rhythmic components in genome-scale data sets. Journal of biological rhythms 25(5):372-380.

Klein, D. C., and R. Y. Moore. 1991. Suprachiasmatic nucleus: the mind's clock. Oxford University Press, Oxford, United Kingdom.

Kreitzman, L., and R. Foster. 2005. Rhythms of Life: The Biological Clocks That Control the Daily Lives of Every Living Thing. Yale University Press, Connecticut, New Haven, USA.

Lafferty, K. D. 1999. The evolution of trophic transmission. Parasitology Today 15(3):111-115.

Lafferty, K. D., and A. K. Morris. 1996. Altered behavior of parasitized killifish increases susceptibility to predation by bird final hosts. Ecology 77(5):1390-1397.

Laland, K. N. 2004. Extending the extended phenotype. Biology and Philosophy 19(3):313-325.

Lee, C., Bae, K., and I. Edery. 1999. PER and TIM Inhibit the DNA Binding Activity of a Drosophila CLOCK-CYC/dBMAL1 Heterodimer without Disrupting Formation of the Heterodimer: a Basis for Circadian Transcription. Molecular and cellular biology 19(8):5316-5325.

Lomb, N. R. 1976. Least-squares frequency analysis of unequally spaced data. Astrophysics and space science 39(2):447-462.

Lundkvist, G. B., Kristensson, K., and M. Bentivoglio. 2004. Why trypanosomes cause sleeping sickness. Physiology 19(4):198-206.

Martinez-Bakker, M., and B. Helm. 2015. The influence of biological rhythms on host–parasite interactions. Trends in ecology & evolution 30(6):314-326.

McDonald, M. J., and M. Rosbash. 2001. Microarray analysis and organization of circadian gene expression in Drosophila. Cell 107(5):567-578.

Moore, J. 1983. Responses of an avian predator and its isopod prey to an acanthocephalan parasite. Ecology 64(5):1000-1015.

Moore, J. 1984. Altered Behavioral Responses in Intermediate Hosts--An Acanthoceptalan Parasite Strategy. The American Naturalist 123(4):572-577.

Moore, J. 2002. Parasites and the behavior of animals. Oxford University Press, Oxford, United Kingdom.

Naylor, E., and B. G. Williams. 1968. Effects of eyestalk removal on rhythmic locomotor activity in Carcinus. Journal of Experimental Biology 49(1):107-116.

Naylor, E. 2010. Chronobiology of marine organisms. Cambridge University Press, Cambridge, United Kingdom.

Nickol, B. B., Heard, R. W., and N. F. Smith. 2002. Acanthocephalans from crabs in the southeastern US, with the first intermediate hosts known for Arhythmorhynchus frassoni and Hexaglandula corynosoma. Journal of Parasitology 88(1):79-83.

Poulin, R. 2010. Parasite manipulation of host behavior: an update and frequently asked questions. Advances in the Study of Behavior 41:151-186.

SAS Institute. 2015. JMP 12. SAS Institute Inc., Cary, North Carolina, USA.

Scargle, J. D. 1982. Studies in astronomical time series analysis. II-Statistical aspects of spectral analysis of unevenly spaced data. The Astrophysical Journal 263:835-853.

Schaffer, R., Landgraf, J., Accerbi, M., Simon, V., Larson, M., and E. Wisman. 2001. Microarray analysis of diurnal and circadian-regulated genes in Arabidopsis. The Plant Cell 13(1):113-123.

Schneider, C. A.; Rasband, W. S. and K. W. Eliceiri. 2012. NIH Image to ImageJ: 25 years of image analysis. Nature methods 9(7):671-675.

Sharma, V. K. 2003. Adaptive significance of circadian clocks. Chronobiology international 20(6):901-919.

Shaw, J. C., Korzan, W. J., Carpenter, R. E., Kuris, A. M., Lafferty, K. D., Summers, C. H., and Ø Øverli. 2009. Parasite manipulation of brain monoamines in California killifish (Fundulus parvipinnis) by the trematode Euhaplorchis californiensis. Proceedings of the Royal Society of London Biological Sciences 276(1659):1137-1146.

Tessmar-Raible, K., Raible, F., and E. Arboleda. 2011. Another place, another timer: marine species and the rhythms of life. Bioessays 33(3):165-172.

Thomas, F., and R. Poulin. 1998. Manipulation of a mollusc by a trophically transmitted parasite: convergent evolution or phylogenetic inheritance? Parasitology 116(05):431-436.

Thomas, F., Adamo, S., and J. Moore. 2005. Parasitic manipulation: where are we and where should we go?. Behavioural processes 68(3) :185-199.

Varaldi, J., Petit, S., Boulétreau, M., and F. Fleury. 2006. The virus infecting the parasitoid Leptopilina boulardi exerts a specific action on superparasitism behaviour. Parasitology 132(06):747-756.

Wager-Smith, K., and S. A. Kay. 2000. Circadian rhythm genetics: from flies to mice to humans. Nature genetics 26(1):23-27.

Wesołowska, W., and T. Wesołowski. 2014. Do Leucochloridium sporocysts manipulate the behaviour of their snail hosts?. Journal of Zoology 292(3):151-155.

White, A. Q. 1976. Rhythmic aspects of the behavior of an intertidal sandy beach organism: Emerita talpoida. PhD thesis, University of South Carolina, Columbia, USA.

Figures and Tables

Table 1. Summary of results for a logistic regression model for the probability of a given *E. talpoida* individual being arrhythmic. Of the various predictor variables, log transformed trematode load showed the greatest significance. N = 236.

Term	Estimate	SE	ChiSq	P-value
Intercept	-0.221	1.127	0.039	0.844
Log (N_{acan} + 0.1)	-0.087	0.134	0.420	0.517
Log (N_{tre} + 0.1)	-0.230	0.121	3.737	0.053
Month Collected (May)	0.471	0.314	2.270	0.132
Month Collected (Jun)	-0.244	0.213	1.342	0.247
Sex (Female)	-0.167	0.415	0.165	0.685
Ovigerity (No)	0.077	0.174	0.197	0.657
Carapace Length (mm)	0.033	0.045	0.525	0.469
ChiSq = 6.7094, *df* = 7, *P* < 0.01, n =236				

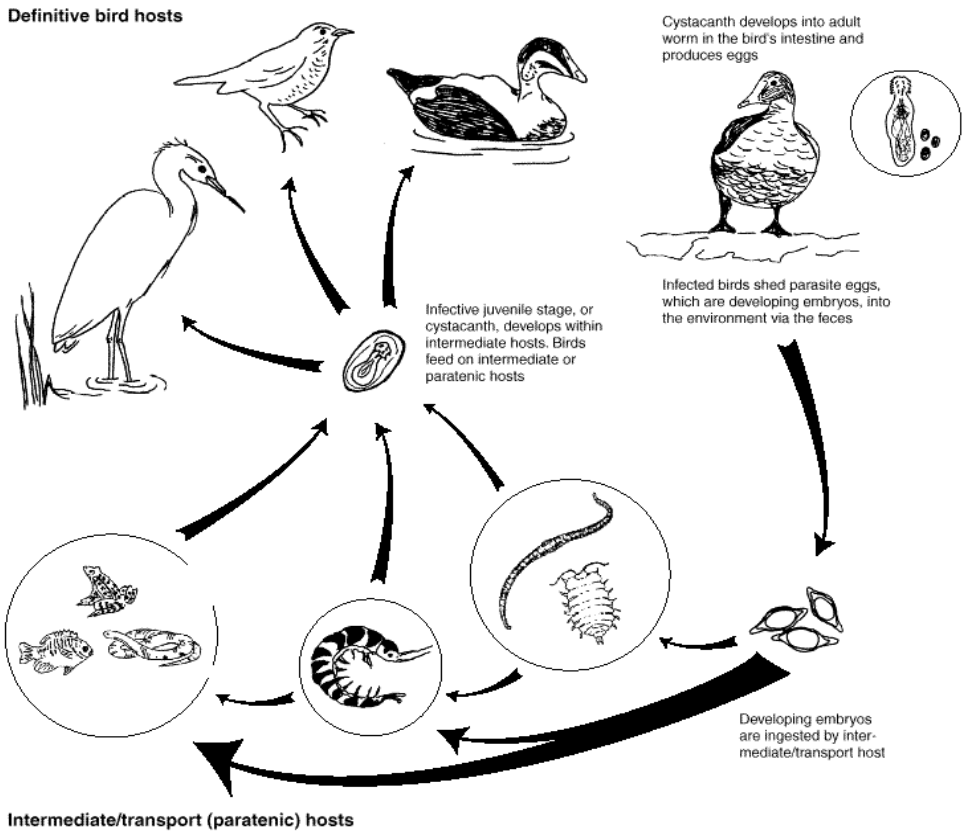

Figure 1 – Life Cycle of Acanthocephalans (retrieved from Friend and Franson 1999). Thorny-headed worms undergo a complex life cycle characterized by both intermediate and definitive hosts. The intermediate hosts are infected by developing embryos through ingestion while definitive hosts are infected by cystacanths (infective juvenile stage) when they prey upon intermediate hosts.

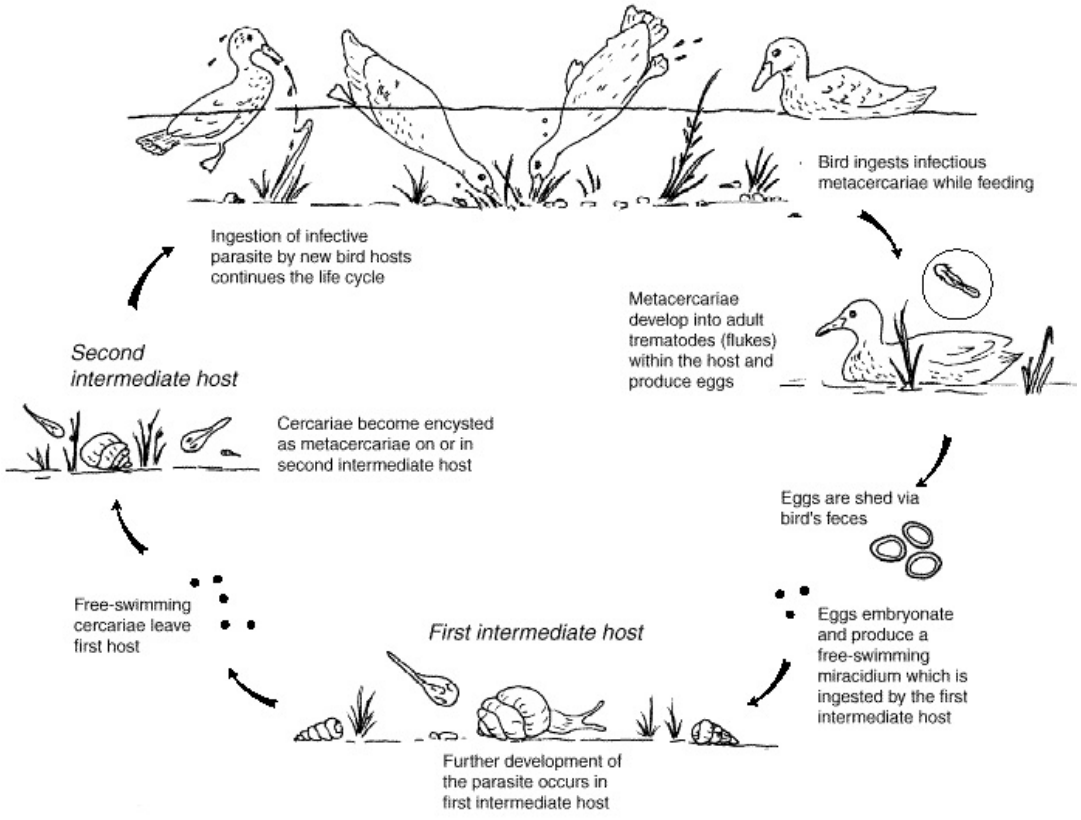

Figure 2 – Generic Life Cycle of Trematodes (retrieved from Friend and Franson 1999). Trematodes undergo a complex life cycle characterized by multiple stages of intermediate hosts and a final definitive host. Free-swimming miracidium hatches out from trematode eggs and enters the first intermediate host through ingestion. After undergoing development, they leave the first host has free-swimming cercariae and infect the second intermediate host where they encyst as metacercariae. Through predation, these metacercariae are passed on to a definitive host where they develop into adult trematodes and produce eggs.

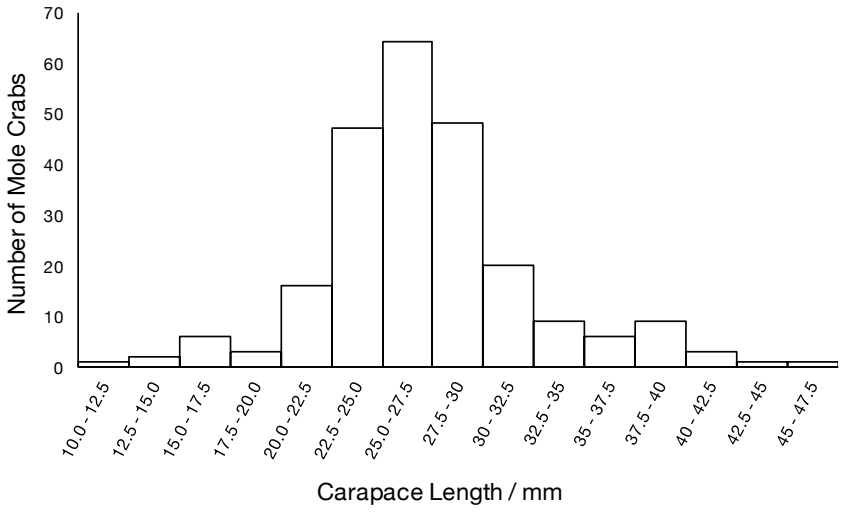

Figure 3 – Unimodal size distribution of *E. talpoida* samples collected and analyzed (n = 236). Carapace length of the majority of sampled individuals fell between 20 to 32.5 mm.

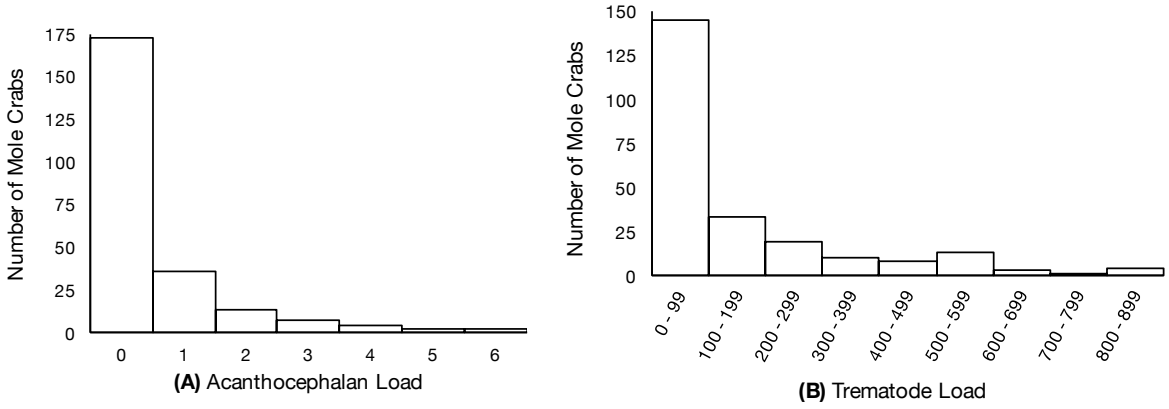

Figure 4 – Skewed distribution of Acanthocephalan load (A) and Trematode Load (B) (n = 236). Majority of sampled mole crabs showed low infection levels for both *Profilicollis sp.* (Acanthocephalan) and *Microphallus sp.* (Trematode). Note that the number of trematode metacercariae typically outnumbers the the acanthocephalan cystocanths by several orders of magnitude.

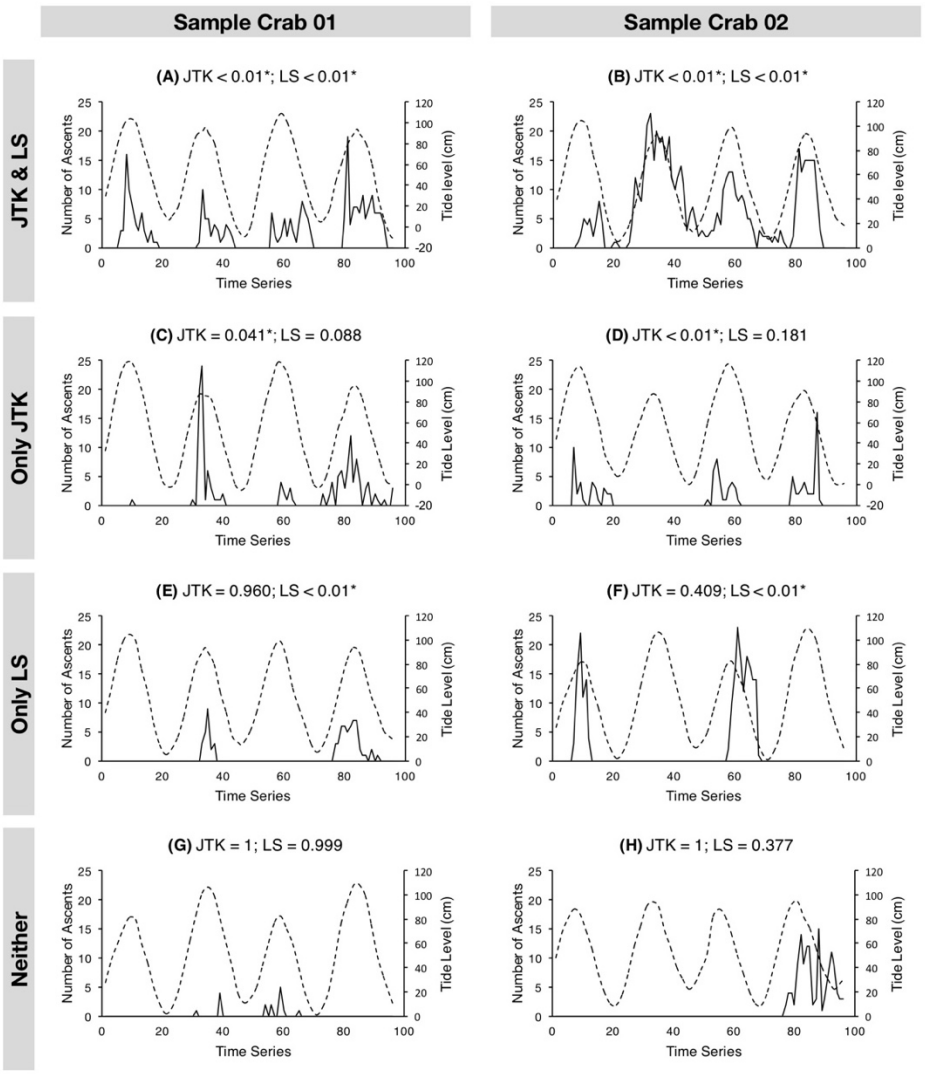

Figure 5 – Rhythmic Activity Assay Graphs for Selected Crabs and their associated P-value output from the JTK_CYCLE (JTK) and Lomb-Scargle (LS) algorithm. Each individual graph represents the activity levels of a selected crab over a 48h period. Dotted lines represent the tide level and solid lines represent the number of ascent per 0.5-hour interval. Each unit on the time series corresponds to a 0.5-hour interval. Asterisks indicate significance at the 95% confidence level. Pairs corresponding to differential assignments by the JTK and LS algorithms were chosen. Samples A and B were designated as rhythmic by both algorithms; Samples C and D were designated rhythmic only by JTK; Samples E and F were designated rhythmic only by LS; while Samples G and H were designated rhythmic by neither.

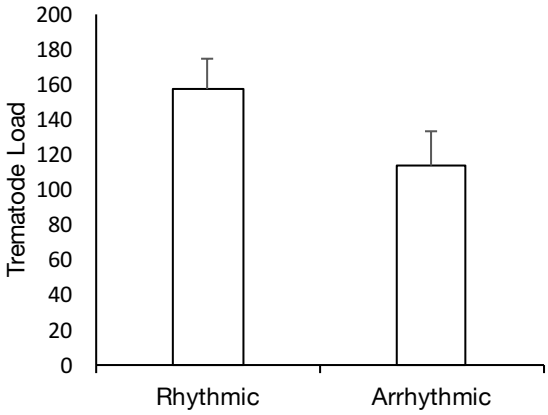

Figure 6 – Higher Trematode load in rhythmic mole crabs. A significantly greater mean trematode load was observed in rhythmic individuals (n = 131) compared to arrhythmic individuals (n = 105) (t = 1.72, df = 219.3, P = 0.044). Load was measured as the number of metacercariae (larval stage of Trematodes) counted in individual mole crabs.

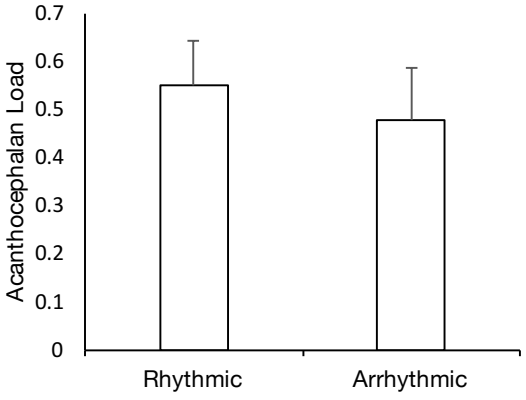

Figure 7 – Similar Acanthocephalan load in rhythmic and arrhythmic mole crabs. The difference between the mean Acanthocephalan load for rhythmic (n = 131) versus arrhythmic (n = 105) individuals was not significant (t = 0.51, df = 215.2, P = 0.304). Load was measured as the number of cystacanths (larval stage of Acanthocephalans) counted in individual mole crabs.