Doctoral Thesis

Host preference of the ectoparasitic isopod *Tachaea chinensis* among freshwater shrimps
外部寄生性等脚類エビノコバンの宿主選択性

Al Wahaibi Mohamed Khalfan

Department of Bioresource Science
Graduate School of Integrated Sciences for Life
Hiroshima University

March 2023

Acknowledgments

I am very grateful to Almighty Allah, for giving me the strength, focus and support during the course of my PhD degree completion. My sincere appreciation goes to the scholarship program provided for me by the Ministry of Education, Culture, Sport, Science and Technology (MEXT) and the continuous support from the Embassy of Japan in Oman and the Embassy of Oman in Japan, without their help and kind support none of this work could have been possible. I am also grateful to all the staff at Sultan Qaboos University and the center of Excellence in Marine Biotechnology for the never-failing assistance and allowing me the opportunity to pursue my doctoral study in Japan. My heartfelt gratitude to my supervisor Dr. Hidetoshi SAITO for his willingness, kindness, unlimited support, valuable comments, supervision and precious time and the graduate school of Integrated Sciences for Life – Hiroshima University for accommodating my research during my study. I would also wish to express my sincere appreciation to my academic advisors: Dr. Koichiro KAWAI, Dr. Tetsuya UMINO and Dr. Masayuki YOSHIDA for their unlimited motivation and kindness during the course of my research. I very grateful to Dr. Wenresti Gallardo -Department of Marine Science and Fisheries, College of Agriculture and Marine Sciences, Sultan Qaboos University- for the continuous guidance, the valuable time and sincere advice at all times. Special thanks to all my friends at the department of Bioresource science- Laboratory of Aquatic Ecology, for the warmhearted welcome, wonderful experiences, and outstanding support. My life in Japan couldn’t be more unique without your presence. I would like to thank the student support office, university coop and all the departments responsible for students’ life at Hiroshima university for making my stay so much easier with their continuous help and recommendations throughout my life in Japan. Finally I would like to thank my loving parents, family and friends, who supported and always believed in me. I am very grateful to you all.

Table of contents

List of Abbreviations

P. paucidens	*Palaemon paucidens*
P. sinensis	*Palaemon sinensis*
T. chinensis	*Tachaea chinensis*
GW	Gonads weight
HW	Hepatopancreas weight
GSI	Gonadosomatic index
HSI	hepatosomatic index
CF	Condition Factor
K_n	Relative condition factor

List of Figures

List of Tables

Chapter 1

General Introduction

1.1 General background

Isopods are a diverse group of animals including more than 10,000 species (Wilson 2008). The order Isopoda is a home to nine suborders that can be found in various habitats; from the deep ocean's areas to the terrestrial mountainous regions (Nagler et al. 2017). Although they are known by the dorsoventrally flattened body, other species (e.g., deep sea species) could greatly diverge from this typical body plan (Wilson 2008). Within the recorded 942 terrestrial water (e.g., ponds, rivers and glaciers) isopods, there are a wide range of life styles. These includes carnivores, scavenger-carnivores, herbivores, omnivores and detritivores-omnivores life style isopods (Wilson 2008).

The suborder Flabellifera contain 13 known families. Out of which five families that are relatively similar (Cirolanidae, Tridentellidae, Corallanidae, Aegidae and Cymothoidae) (Delaney 1989). They can be found in association with fish and crustaceans (Sikkel and Welicky 2019). Demonstrating a wide range of mode of living; from being temporary parasitic to permanent endo-parasitism (Tanaka 2007).

The family Corallanidae is a home to both parasitic and free-living species of isopods (Delaney 1989). Although they are temporary parasites (often leave the host after feeding for reproduction), they are usually categorized as free-living parasites (Sikkel and Welicky 2019). Corallanidae contains 6 genera with 67 described species (Delaney 1989). Species of the genus *Tachaea* are found exclusively in fresh water environment, containing 8 species that are known to associate with fresh water shrimps and fish but restricted to the Indo-west pacific region (Mariappan et al. 2003; Delaney 1989). For example, *T. caridophaga* and *T. picta* has been found infesting on fresh water shrimps from the families Palaemonidae and Atyidae in Australia (Riek 1967; Song and Min 2018). While *T. crassipes* and *T. lacustris* are found inhabiting brackish water environments in Singapore and Indonesia respectively (Bruce 2004; Bowman and Botosaneanu 1992). *T. spongillicola* has been reported in fish, shrimps and sponges (*S. carteria* and *S. Lacustris)* in India (Mariappan et al. 2003; Sahoo et al. 2022). While *T. tonlesapensis* and *T. koreaensis* sp. n. were found infesting on shrimps in Cambodia and South Korea respectively (Nunomura 2006; Song and Min 2018). *T. chinensis* is found infesting freshwater shrimps and prawns in Japan, China, Vietnam, Thailand and Malaysia (Nagasawa et al. 2018).

T. chinensis is first reported in 1910 infesting freshwater shrimps. However, it can be found in saline as well as freshwater environments. The strong adaptability to different environmental conditions, especially salinity, is believed to aid in the prevalence of this isopod in nature (Xu et al. 2019). Taxonomically, they distinguished from the other species in the same genus by having the Propodus of first pereopod being expanded, the Maxilliped palp has 3 articles, and the endopod of the uropod surpasses the pleotelson (Song and Min 2018). It bears seven pairs of pereopods and four pairs of pleopods. Seemingly these structures allow for the capability of propelling and swimming in this isopod (Li et al. 2021). The mouth parts are greatly modified into blade like structures, that could easily penetrate the body of the host while preventing any blood loss from the process. It is also equipped with 6 salivary glands around the mouth which could help in the secretion of several anti-inflammatory and anti-immunomodulatory compounds during infection (Li et al. 2019a).

The genus *Tachaea* has the highest percentage of temporary parasitism among the 6 genera in the family Corallindae (Delaney 1989). It is assumed that *T. chinensis* would feed on the host at younger stages, while it might detach for reproduction during mature stages (Ota 2019). Which is also supported by Xu et al. (2021), who found that manca stage *T. chinensis* became progressively weak and subsequently died after the sixth day in the absence of host (e.g., prey).

Isopods has internal fertilization, in which subsequently the embryos being held in the marsupium (Wilson 1991 and 2007). According to Xu et al. (2021), the number of mancae ranges between 31 to 86 per brood in the *T. chinensis* isopods. Undergoing five distinct embryonic stages within 30 – 38 days. Although the complete life cycle could span, on average, 180 days (Figure 1.1).

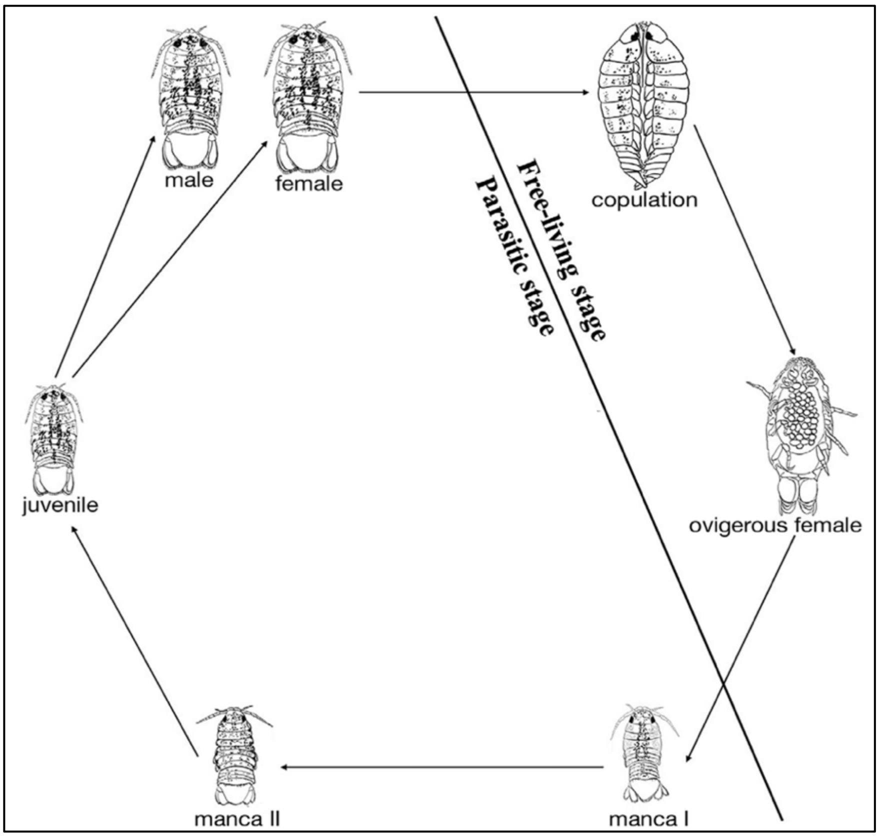


Figure 1.1: The complete life cycle of *T. chinensis* under laboratory condition (Xu et al. 2021).

The distribution pattern of isopods is usually governed by its host specificity. *T. chinensis* was found infesting several freshwater shrimps and prawns in eastern Asia (Nagasawa et al. 2018). In China, it is hypothesized that the low competition from other parasitic isopods might be responsible for the increased number of potential hosts for *T. chinensis* (Xu et al. 2019). While in Japan, the distribution of these isopods was found to be governed by the type of the host shrimps available at a given location (Nagasawa et al. 2018; Ota 2019). *T. chinensis* has been found infesting several genera, including; *Caridina*, *Neocaridina*, *Paratya*, *Macrobrachium*, *Palaemon*, and *Penaeus*. However, until now, whether *T. chinensis* isopods exhibits host selection and/or size preference abilities to choose from these freshwater host shrimps remained unclear. Host size preference could play an important role in the prevalence and survival of the isopods. Although studies on the size preference of the isopod is scarce, Ota (2019), found that the relatively small *Neocaridina* spp. did not provide enough space for larger *T. chinensis* to attach. Indicating that, the isopod might change host species/size preference according to its growth stage. Whether or not parasites grow in accordance to the body size of its host depend on the space available for it to attach. Therefore, host body size and species could also limit the evolutionary size trend of a given parasite (Tsai et al. 2001; Pawluk et al. 2014). Further research on the aspects of size and host specificity is needed to understand the preference of *T. chinensis.*

Crustaceans are known as one of the most taxonomically diverse groups in regards to habitat selection and the use of chemical, tactile and visual communication sensory systems (Hebets and Rundus 2010). These complex systems are used for various purposes, including: predator avoidance, habitat selection, sex recognition and mate selection (Chiussi and Diaz 2002; Huang et al. 2005; Acquistapace et al. 2002; Cook and Munguia 2013; Kamio et al. 2008 and Aquiloni and Gherardi 2008). Parasitic crustacean also demonstrates sensory modalities and mechanical strategies during both early life stages and mature stages. For example, the cymothoid isopods has been shown to utilize both visual and chemical senses to detect suitable hosts (Cook and Munguia 2013). While light detection was also suggested in the bopyrid isopods, necessary for breeding purposes (Beck 1980a). The diverse modes of living and the complex sensory systems possessed by isopods may raises the question about the mechanism behind the initial search and selection of appropriate hosts in nature.

The study of how parasitic isopods locate and choose suitable host can help understand the population dynamics of these isopods in nature (Cook and Munguia 2013). Moreover, it sheds light on how such associations is maintained, which ultimately help in conservation of species and its habitats (Olabarria et al. 2002). Choice experiments are often used to investigated the preference of animals for a certain food and/or habitats (Limviriyakul et al. 2016). Results from these experiments could provide important mechanical understanding of their behavior and selection pattern in nature (Olabarria et al. 2002). In addition, behavioral experiments could also offer insights on the movement behavior and the complexity of infestation strategy used by the isopods at different life stages (Mancinelli 2010; Cook and Munguia 2013). Hence, the establishment of better management and risk mitigation measures.

Parasitic isopods cause numerous effects to the hosts. These include: blood loss, pathogen transport, osmoregulatory deficiency and/or reproductive inhibition (Rameshkumar and Ravichandran 2014). Upon attachment, isopods usually cause localized inflammation on the host, however serious damages will be noticed over the long-term (Rameshkumar and Ravichandran 2013). Isopods also found to affect the growth and gonadal development of its associated host. For example, the isopod *Probopyrus pandalicola* has been shown to cause nutrient drain and hormonal interference on the caridean shrimp *Palaemonetes paludosus*. An interference that could ultimately lead to the inhibition of ovarian development and/or fecundity reduction in female shrimps (Beck 1980b; Dumbauld et al. 2011). *T. chinensis* has been found to interfere with the antioxidant and the immune response of *Macrobrachium* *nipponense* (Li et al. 2020). Further effects of this isopod include stressing the glucose metabolism and the potential secretion of anti-clogging and anti-inflammatory molecules to facilitate the steady supply of blood from its associated host (Li et al. 2019a; 2019b). Other research on this regard, hypothesized the potential effect of *T. chinensis* on the visual system of the host (Yu et al. 2021). Moreover, the holes caused by these isopods were found to attract pathogens such as; *Vibrio* sp. (Ueki et al. 1988). However, until now information on the effect of this parasite on the growth and reproduction of these freshwater shrimps is still unclear.

Freshwater caridean shrimps are ecologically important and resemble an important component of the livelihood of many freshwater communities. Despite their use in the ornamental industry and as fishing baits, they play an important role in determining benthic community composition and aquatic insects' biomass (March et al. 2002). However, among the 655 described species, 13 are currently regarded as endangered (Grave et al. 2007). Therefore, *T. chinensis* could potentially place another heavy burden on these shrimps along with overexploitation (Puspitasari et al. 2013).

The hepatopancreas play an important role in enzymes secretion, food absorption and transport and lipid storage in decapods crustaceans (Felgenhauer 1992). An organ that is found to be challenged during infection by parasitic isopods (Yu et al. 2021). The gonadosomatic index (GSI) and hepatosomatic index (HSI) are effective measures to determine the breeding cycle of an organism (Puspitasari et al. 2013). It evaluates the gonads /hepatopancreases mass as a proportion of the total body mass (Barber and Blake 2006). Other available indices included the condition factor, which evaluates the overall organism health (Rochet 2000). Parasitic infection usually cause swelling and leads to female infertility in shrimps (Gopalakrishnan et al. 2017). Therefore, measurements of GSI and HSI could provide an important insight on the effect of parasitism on the gametogenic cycle of shrimps (Barber and Blake 2006). The Lake prawn *Palaemon paucidens* reproductive traits were found to be related to fluctuation of water temperatures. In which the peak in GSI and the breading period were observed during higher water temperatures (Puspitasari et al. 2013). However, Despite the presence of studies on reproduction and reproductive output of *Palaemon paucidens*, information on whether an alteration to be produced by the presence of the parasitic isopods *T. chinensis* remain unknown.

1.2 Study Objectives

The aims of the present study are:

1) To determine the host–parasite size relationship between *Tachaea chinensis* and common Caridean host shrimps in the natural environment.
2) To investigate the size selection behavior of *Tachaea chinensis* isopods across common host shrimps in laboratory condition.
3) To identify the degree of host specificity of *Tachaea chinensis* isopods in laboratory condition.
4) To understand the effects imposed by *Tachaea chinensis* isopods on growth and gonadal development of the Lake Prawn *Palaemon paucidens*.

1.3 Thesis overview

The present thesis is divided into six chapters:

Chapter 1: General Introduction. This chapter provides the background information, outlines the objectives and describes the structure of the study.

Chapter 2: Size Relationship of the Ectoparasite *Tachaea chinensis* in the Host Shrimp *Palaemon paucidens* From Shiga Prefecture, Japan. In this chapter, the ratios between carapace length and body length of *Tachaea chinensis* and common Caridean host shrimps (*Palaemon paucidens* and *Neocardina* spp.), was studied using wild specimens.

Chapter 3: Host size selection in the ectoparasite *Tachaea chinensis* (Isopoda: Corallanidae) under laboratory condition. In this chapter, the size preference of *Tachaea chinensis* isopods was further examined under laboratory conditions.

Chapter 4: Host species selection of *Tachaea chinensis* (Isopoda: Corallanidae) and host-parasite predation under laboratory conditions. This chapter tested the host specificity *Tachaea chinensis* isopods using pairwise choice experiment and investigated the possibility of predation of the isopod by its hosts.

Chapter 5: Effects of the ectoparasite *Tachaea chinensis* on the growth condition and gonads development of the freshwater shrimp *Palaemon paucidens.* This chapter provides insights on the effect caused by *Tachaea chinensis* isopods by means of comparing the host growth pattern and the reproductive development between infected and non-infected host shrimps.

Chapter 6: Overall discussions. The overall discussion summarizes the main finding of the whole study and addresses the main conclusions.

Chapter 2

Size Relationship of the Ectoparasite *Tachaea chinensis* in the Host Shrimp *Palaemon paucidens* From Shiga Prefecture, Japan

2.1 Introduction

There are nine described species in the genus *Tachaea*- family corallanidae, in which one species (e.g., *Tachaea chinensis*) is found in Japan (Nunomura and Shimomura 2020). These parasitic isopods are known as symbionts of freshwater shrimps and prawns. For example, *Tachaea koreaensis* Song and Min, 2018 is an ectoparasite of *Macrobrachium nipponense* De Man, 1849 and *Palaemon paucidens* De Hann, 1844 shrimps in South Korea (Song and Min 2018). In India, *Tachaea spongillicola* Stebbing, 1907 is found attaching on the carapace of *Macrobrachium nobilii* Henderson and Matthai, 1910 (Mariappan et al. 2003). Previous research concerning these isopods were mainly focused on the taxonomy, morphology, pathology and the immunology of the species (Nunomura 2006; Song and Min 2018; Nunomura and Shimomur 2020; Li et al. 2021; Ueki et al. 1988; Li et al. 2018). While others, shed light on the structure and the functionality of their mouthpart's appendages. whereby it is indicated that the overall formation and the blade-like structures found in the mouth of these isopods facilitate in the cutting and blood sucking behaviour during attachment (Li et al. 2021). However, the difficulties in obtaining enough specimens of this ectoparasite has been a major bottleneck in fully understanding the dynamics of their populations (Ota 2019).

The temporary ectoparasite *Tachaea chinensis* Thielemann, 1910, is widely distributed in Eastern Asia (Shiino 1965; Saito 2011; Nagasawa et al. 2018; Xu et al. 2019, 2021; Nunomura and Shimomura 2020). It is often characterized by the broad range of potential hosts and its remarkable adaptability in different saline and freshwater habitats. Presumably, assisting in the observed wide distribution and prevalence in lacks, rivers and brackish water environments (Xu et al. 2019). Despite the presence of ecological surveys on the isopod *Tachaea chinensis*, Information on the host selectivity and host-parasite interactions in regard to size preference are yet very scarce. In one study on the biphasic life cycle of *Tachaea chinensis*, they found that the young isopods appeared in autumn and spring while it might switch to a free-living adult state, likely for reproduction, in summer (Ota 2019). The same study also indicated that, concrete information on the fate of large sized *Tachaea chinensis* (> 6 mm) is still lacking, as they were rarely found on small host shrimps (e.g., *Neocaridina* spp.). Therefore, further studies on adult stage *Tachaea chinensis* is needed to fully understand the isopods infestation pattern and preference.

Palaemon paucidens shrimps are widely distributed in the Japanese freshwater environment (Toyota and Seki 2014). Recently, a relatively large *Tachaea chinensis* isopod was found attaching on the carapace of this shrimp in a stream flowing into Lake Biwa in central Japan. Hence, host size could resemble a determinant factor for growth and the maximum size to be attained by these isopods in nature, although further research is needed on this regard in the future.

The current study aimed to determine the prevalence and the ectoparasitic period of *Tachaea chinensis* on the host shrimp *P. paucidens*. Whereby, we place special emphasis on the size selection aspect of this host-parasite interaction in nature.

2.2 Materials and methods

2.2.1 Parasite and host shrimps' collection

Monthly samples were obtained from an agricultural canal in the Etsura river stream flowing into lake Biwa, Hikone City, Shiga Prefecture, Japan (35º14′55.7″N 136º12′46.4″E). The sampling period extended from April 2018 to December 2019. Host shrimps and *T. chinensis* isopods were scooped from an area, approximately 50 m^2, around submerged aquatic plants (e.g., *Egeria densa* Planch, 1849) and near river beds. Scooping was done using hand nets (2.5-mm mesh, opening 35×35 cm, handle length 240 cm). All specimens were then fixed in 70% ethanol for later transportation and subsequent analysis. Water temperature was measured, on-site, using a water quality monitor LAQUAact D-74 (HORIBA Scientific, Japan) during each sampling.

2.2.2 Staging and measurements

In the laboratory, host shrimps were identified to the species level following Toyota and Seki (2014), except for *Neocaridina* species, in which complex unidentified mixed species are present, taxonomical identification was made until the genus level. *T. chinensis* isopods were identified as Manca, if individuals were lacking the 7th pair of pereiopods. They were classified as two-phase molt isopods, when individuals are at half way of the molting process and having enlarged posterior body. Immature stage isopods were known by the presence of the all seven pairs of walking legs but lacking the developed penes (Males) or brood pouch (Females). Finally, the adult stage isopod, which is identified by the presence of a fully developed male penes or female brood pouch (Ota 2019). The body length of parasites was measured from the frontal border of the head to the posterior margin of the pleotelson. While the carapace length of host shrimps was measured from the orbital edge to the posterior margin of the carapace.

2.2.3 Statistical analysis

Initial analysis using two-tailed parametric tests were performed, however, upon the unfulfillment of the homogeneity of error variance and the normality assumptions, non-parametric equivalent tests were used. Using a simple linear regression and the least square method- the relationship between the parasite body length and the carapace length of host shrimp were analysed. The differences between the body lengths of *T. chinensis* and the carapace lengths of the hosts was validated using the Welch's *t*-test and Mann-Whitney *U*-tests. All of the statistical analysis were conducted using R statistical software (Version 4.0.3, R Foundation for Statistical Computing, Vienna, Austria) and Excel software (Version 16.57, 2019).

2.3 Results

2.3.1 Prevalence of *T. chinensis* on host shrimp

The number of obtained samples for each shrimp species and the calculated infestation prevalence of *T. chinensis* are shown in Tables 2.1 and 2.2. The dominance of *P. paucidens* and *Neocaridina* spp. host shrimps ranged between 48.2–93.6% (mean ± SD 76.1 ± 13.9%) and 6.4–51.8% (23.9 ± 13.9%), respectively. In total 944 *P. paucidens* and 279 *Neocaridina* spp. were sampled in this study. Except for the latter host shrimps, no other shrimp species was targeted. In regard to *T. chinensis*, a total of 181 isopods were retrieved from *P. paucidens* and 22 specimens were obtained from *Neocaridina* spp. The prevalence of *T. chinensis* scored 45.5% (23.1 ± 13.7%) on *P. paucidens* shrimps, which later increased in the months of September to December. However, it decreased in June and was not detected in July 2018 and 2019. On the other hand, monthly prevalence on *Neocaridina* spp. reached 28.6% (6.4 ± 10.9%), but were only restricted to the months of August–September in both years.

2.3.2 Seasonal variation in body length of *T. chinensis* from host shrimps

T. chinensis measurements of the minimum, maximum and the size frequency distribution are shown in Tables 2.1, 2.2 and Figure 2.1. On one hand, *P. paucidens* specimens revealed three mancae (body length 2.0–2.6 mm) and 178 immature isopods (3.2–10.3 mm). On the other hand, examination on *Neocaridina* spp. specimens revealed four mancae (2.0–2.4 mm) and 18 immature individuals (2.7–5.8 mm). Adults *T. chinensis* was absent from either host taxa during this study. Three mancae (2.0–2.4 mm) were found at the two-phase molt stage, while only one on immature individual (3.6 mm) were observed infesting the *Neocaridina* spp. host shrimp. In April 2018, infestation on *P. paucidens* was first noticed, in which *Tachaea chinensis* had mean length of 6.0 mm (max. 7.0 mm). Which later reached a mean length of 7.2 mm (max. 9.4 mm) in June 2018. No infestation was recorded in July 2018.

However, the infestation appeared again in August 2018 scoring a mean length of 4.1 mm (min. 3.2 mm) and the regular sightings continued until June 2019 (9.1 mm (max. 10.3 mm)). The infestation was not present in July 2019, though it appeared again in August 2019, therefore displaying an infestation scheme similar to that of the previous year. Examination of *Neocaridina* spp. samples revealed a single *T. chinensis* (body length 3.6 mm) obtained in August 2018. In the following month the mean length of the parasites increased to 4.0 mm (max. 4.3 mm).

Records of infestation was not observed between October 2018 and July 2019, although Manca stage isopods appeared once more in August 2019, reaching a mean length of 4.8 mm (max. 5.8 mm) in October 2019. A single *T. chinensis* (body length 5.1 mm) was retrieved from *Neocaridina* spp. in November 2019. There was no significant difference between the body lengths of *T. chinensis* obtained from *P. paucidens* and *Neocaridina* spp. in August 2019 (*t*-test, $P > 0.05$). However, the lengths differ significantly between the two host shrimps in September 2019 (*t*-test, $P < 0.01$).

The water temperature records during this study are shown in Tables 2.1 and 2.2. The mean body length of *T. chinensis* rapidly increased during the month from August to September 2018 and from May to June 2019 which are usually associated with water temperatures ranges between 20.2 – 29.5°C. Water temperatures did not vary in October 2018 towards April the following year, in which it fluctuated between 7.4–19.8 °C.

2.3.3 Seasonal variation in carapace length of host shrimps

The proportions of Infested and non-infested *P. paucidens and Neocaridina* spp. in regards to the size frequency distributions are shown in Appendix Figure S1 and S2. Except in May 2019, there was no significant difference between infested and uninfected *P. paucidens* shrimps (*U*-test, $P < 0.05$). Similarly, was observed in *Neocaridina* spp. samples, there was no significant difference between infested and uninfected individuals in regard to size in August and October 2019 (*U*-test, $P > 0.05$). The size frequency distributions of both host shrimp regardless of the infection level (Infested vs non-infested) are shown in Figure 2.2. *P. paucidens* new recruits were found in July 2018 (minimum carapace length 2.4 mm), and progressively attained a mean length of 5.5 – 6.7 mm in September and October 2018 respectively. New recruits of *P. paucidens* re-appeared in July 2019 (min. 3.2 mm), and reached a length of 6.6 mm in October. No significant difference was observed between the two host shrimps in August 2019 (*t*-test, $P > 0.05$). However, the carapace length was found to be significantly different in September 2019 (*U*-test, $P < 0.01$).

2.3.4 Ectoparasite and host shrimp size relationship

The fraction of parasitized host shrimps in relation to the size of the parasites are shown in Figure 2.3. *T. chinensi*s body length ranged from 2.0 to 10.3 mm on *P. paucidens* (carapace size range 3.6 to 10.4 mm). In contrast, the isopod body length ranged from 2.0 to 5.8 mm on *Neocaridina* spp. (carapace size range 3.7 to 6.7 mm). The host selection behavior of *T. chinensis* was found different at different size classes. Isopods whom body length is less than

6 mm were found infesting both host shrimps. Whereas larger isopods (≥ 6 mm) were only found on *P. paucidens* host shrimps. The proportion of infested shrimps by less than 6 mm *T. chinensis* were found non-significant (*U*-test, $P > 0.05$). Similarly, there was no significant different between the ratio of infested small sized (0.86) and large sized (0.90) *P. paucidens* (Figure 2.4). However, a positive correlation was found between *P. paucidens* carapace length and the body length of the associated *T. chinensis* ($r^2 = 0.479$, $P < 0.01$, $n = 181$) (Figure 2.5). The body length to carapace length ratio was found different between *P.paucidens* (0.86) and *Neocaridina* spp. (0.79), (*t*-test, $P > 0.05$).

Table 2.1 : Number of shrimps and *Tachaea chinensis* prevalence on each host shrimp species, from April to December 2018.

		2018								
Host shrimps		Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
Palaemon paucidens	Total number (*n*)	50	54	42	50	63	34	63	41	34
	Dominance (%)	64.1	83.1	77.8	78.1	92.6	82.9	81.8	59.4	63.0
	Infested number (*n*)	10	7	3	0	12	12	14	13	10
	Prevalence (%)	20.0	13.0	7.1	0	19.0	35.3	22.2	31.7	29.4
	Tachaea chinensis (Immature) (*n*)	10	7	3	0	12	12	14	13	11
	Tachaea chinensis (Two-phase molt) (*n*)	0	0	0	0	0	0	0	0	0
	Tachaea chinensis (Manca) (*n*)	0	0	0	0	0	0	0	0	0
	Infestation by two *Tachaea chinensis*[*1]	0	0	0	0	0	0	0	0	1
	Minimum body length of *Tachaea chinensis* (mm)	4.6	4.6	5.4	-	3.2	4.7	5.0	4.0	4.5
	Maximum body length of *Tachaea chinensis* (mm)	7.0	8.4	9.4	-	4.6	6.0	6.4	7.5	7.1
Neocaridina spp.	Total number (*n*)	28	11	12	14	5	7	14	28	20
	Dominance (%)	28	11	12	14	5	7	14	28	20
	Infested number (*n*)	35.9	16.9	22.2	21.9	7.35	17.1	18.2	40.6	37.0
	Prevalence (%)	0	0	0	0	1	2	0	0	0
	Tachaea chinensis (Immature) (*n*)	0	0	0	0	20.0	28.6	0	0	0
	Tachaea chinensis (Two-phase molt) (*n*)	0	0	0	0	1	2	0	0	0
	Tachaea chinensis (Manca) (*n*)	0	0	0	0	1	0	0	0	0
	Infestation by two *Tachaea chinensis*[*1]	0	0	0	0	0	0	0	0	0
	Minimum body length of *Tachaea chinensis*(mm)	0	0	0	0	0	0	0	0	0
	Maximum body length of *Tachaea chinensis*(mm)	-	-	-	-	3.6	3.7	-	-	-
Water temparature (°C)		16.4	19.9	23.7	28.5	29.5	24.3	19.8	15.1	10.3

[*1]: Individual number of the host shrimps infested by two *Tachaea chinensis*, one on each side of their carapace.
[*2]: Mean value

Table 2.2 : Number of shrimps and *Tachaea chinensis* prevalence on each host shrimp species, from January to December 2019.

Host shrimps		2019												
		Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Total
Palaemon paucidens	Total number (*n*)	26	32	38	43	89	70	73	54	25	27	25	11	944
	Dominance (%)	83.9	86.5	86.4	89.6	84.8	90.9	93.6	72	53.2	48.2	71.4	55.0	76.1[*2]
	Infested number (*n*)	7	7	10	10	13	3	0	10	11	10	11	5	178
	Prevalence (%)	26.9	21.9	26.3	23.3	14.6	4.3	0	18.5	44.0	37.0	44.0	45.5	23.1[*2]
	Tachaea chinensis (Immature) (*n*)	7	7	10	10	13	3	0	7	11	10	13	5	178
	Tachaea chinensis (Two-phase molt) (*n*)	0	0	0	0	0	0	0	1	0	0	0	0	1
	Tachaea chinensis (Manca) (*n*)	0	0	0	0	0	0	0	3	0	0	0	0	3
	Infestation by two *Tachaea chinensis*[*1]	0	0	0	0	0	0	0	0	0	0	2	0	3
	Minimum body length of *Tachaea chinensis* (mm)	5.2	5.5	5.0	4.8	5.2	6.8	-	2.0	4.7	4.5	4.2	5.1	4.7[*2]
	Maximum body length of *Tachaea chinensis* (mm)	7.1	6.5	6.9	7.5	8.0	10.3	-	4.7	5.9	6.5	7.5	7.3	7.1[*2]
Neocaridina spp.	Total number (*n*)	5	5	6	5	16	7	5	21	22	29	10	9	279
	Dominance (%)	16.1	13.5	13.6	10.4	15.2	9.1	6.4	28	46.8	51.8	28.6	45.0	23.9[*2]
	Infested number (*n*)	0	0	0	0	0	0	0	6	5	7	1	0	22
	Prevalence (%)	0	0	0	0	0	0	0	28.6	22.7	24.1	10.0	0	6.4[*2]
	Tachaea chinensis (Immature) (*n*)	0	0	0	0	0	0	0	2	5	7	1	0	18
	Tachaea chinensis (Two-phase molt) (*n*)	0	0	0	0	0	0	0	2	0	0	0	0	3
	Tachaea chinensis (Manca) (*n*)	0	0	0	0	0	0	0	4	0	0	0	0	4
	Infestation by two *Tachaea chinensis*[*1]	0	0	0	0	0	0	0	0	0	0	0	0	0
	Minimum body length of *Tachaea chinensis*(mm)	-	-	-	-	-	-	-	2.0	2.7	4.1	5.1	-	3.5[*2]
	Maximum body length of *Tachaea chinensis*(mm)	-	-	-	-	-	-	-	4.3	4.5	5.8	5.1	-	4.6[*2]
Water temparature (°C)		7.4	8.6	10.8	14.2	20.2	23.6	26.6	29.4	26.7	21.1	14.8	10.4	19.1[*2]

[*1] : Individual number of the host shrimps infested by two *Tachaea chinensis*, one on each side of their carapace.

[*2] : Mean value

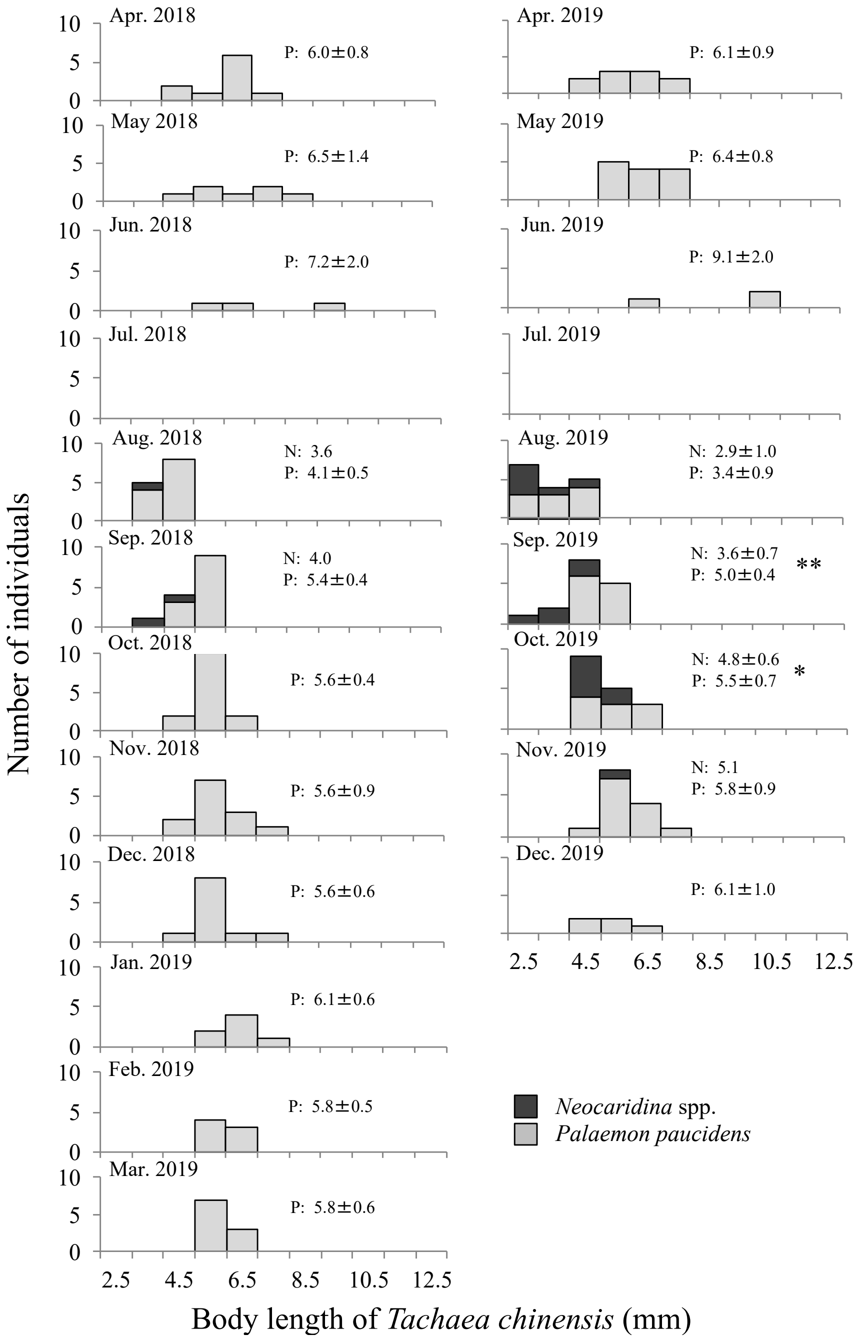


Figure 2.1: Size frequency distribution of *Tachaea chinensis* on the host shrimps. Values are expressed as mean ± standard deviation. N: parasite infestation on *Neocaridina* spp.; P: parasite infestation on *Palaemon paucidens*; *: $P < 0.05$; **: $P < 0.01$ (Welch's *t*-test).

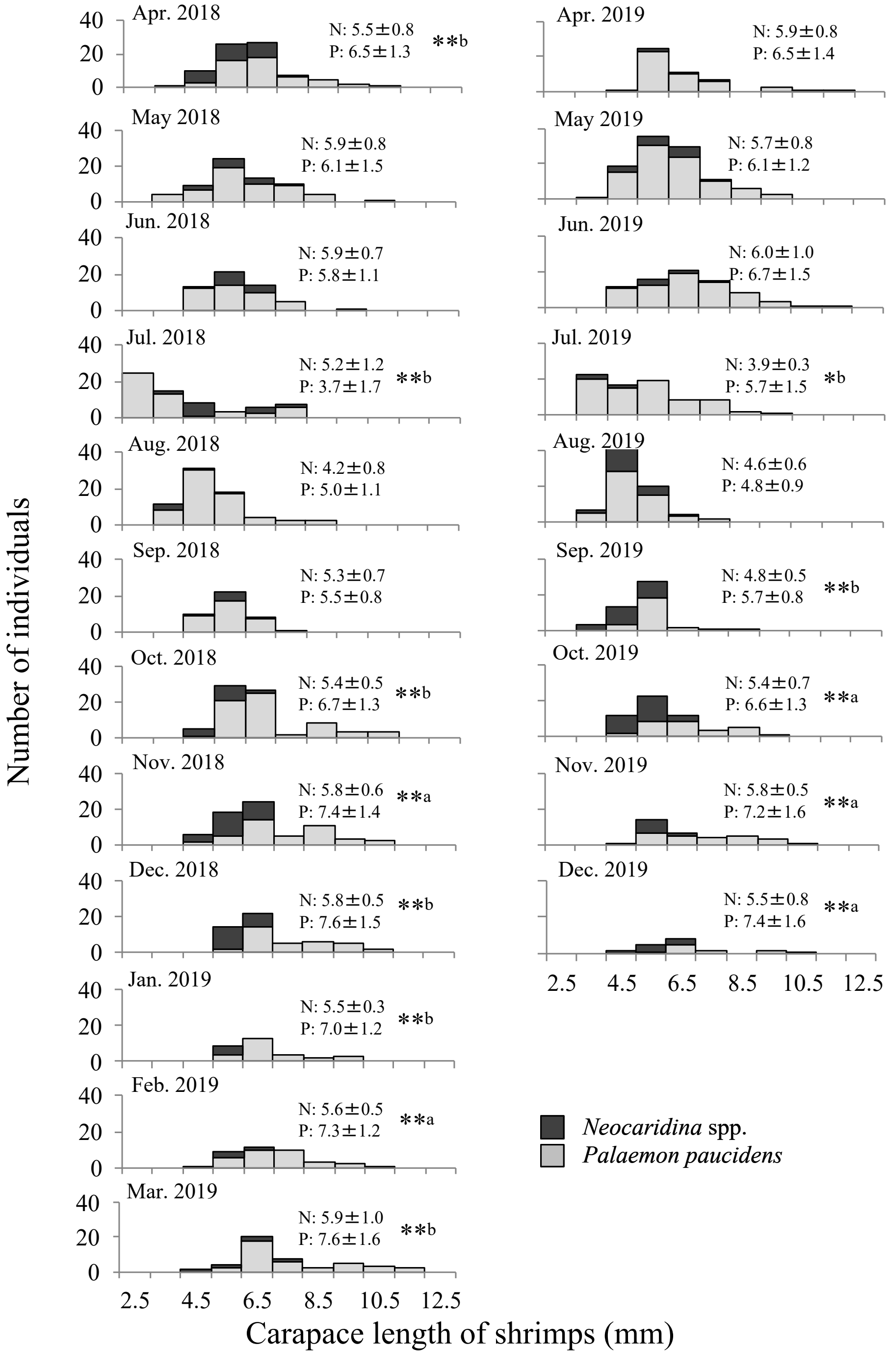


Figure 2.2: Size frequency distribution of the two collected shrimp taxa. Values are expressed as mean ± standard deviation. N: *Neocaridina* spp.; P: *Palaemon paucidens*; [a] Welch's *t*-test, [b] Mann-Whitney *U*-test, *: $P < 0.05$, **: $P < 0.01$.

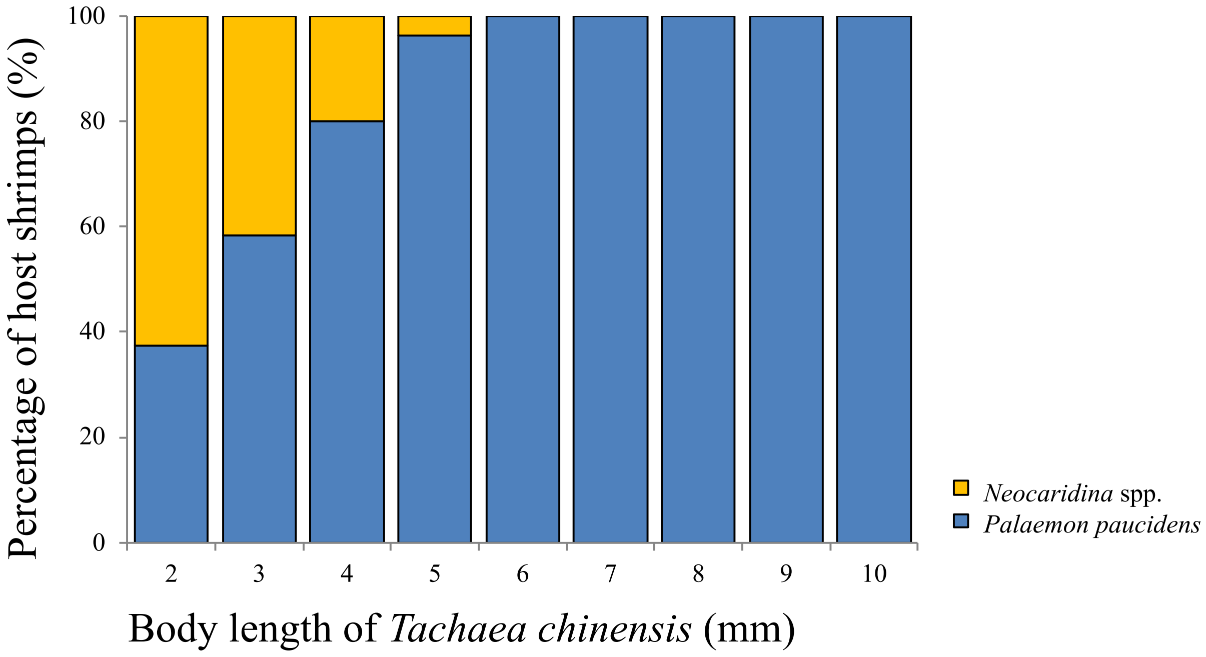


Figure 2.3: Percentage of parasitized host shrimps at different body lengths of *Tachaea chinensis* (*n* = 203).

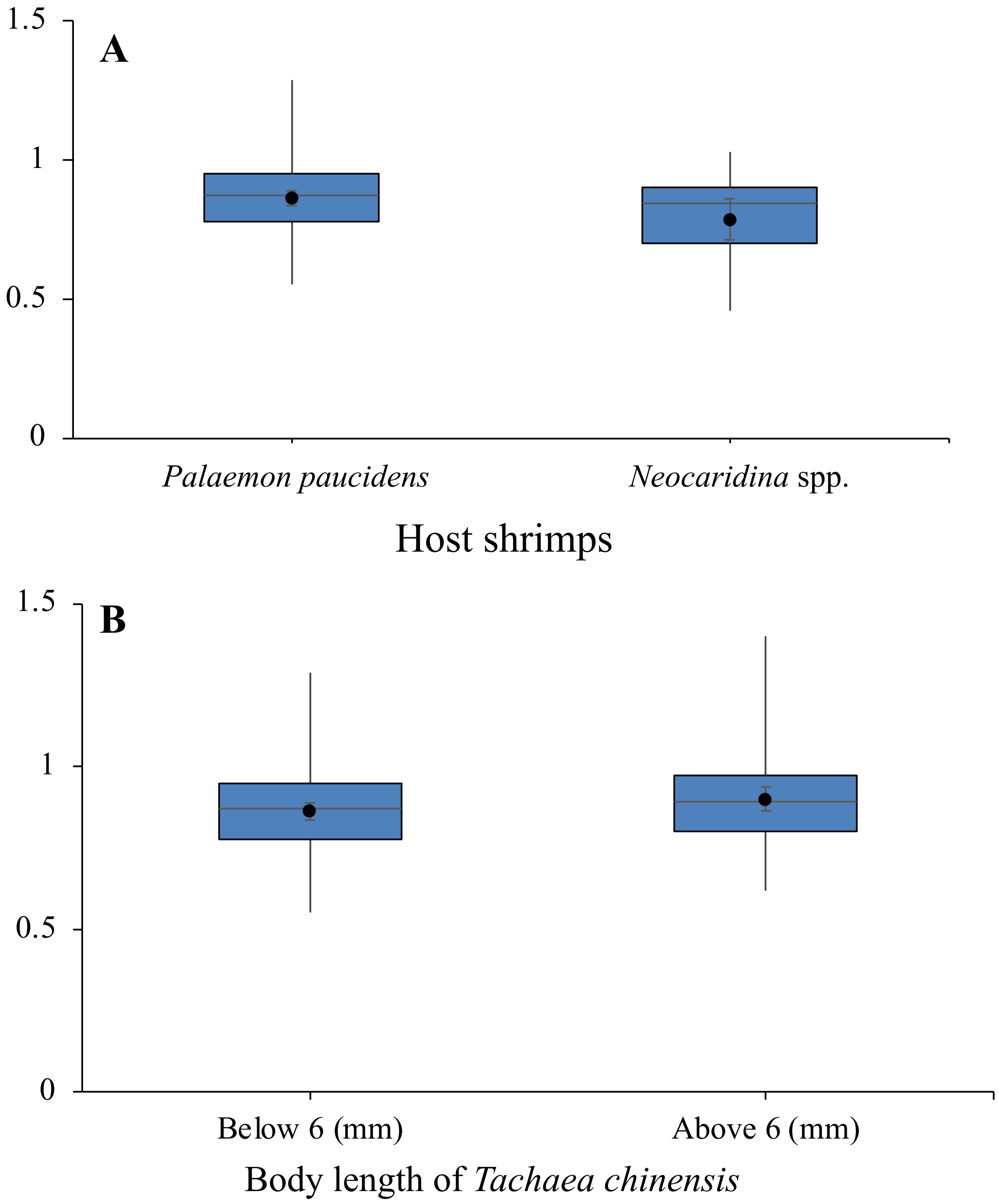


Figure 2.4: Ratios of body length of *Tachaea chinensis* to the carapace length of the host shrimp with regard to host species (*Palaemon paucidens* [*n* = 122] and *Neocaridina* spp. [*n* = 22]) **(A)** and parasites size groups (below [*n* = 122] and larger than [*n* = 59] 6 mm) **(B)**. Boxplots are shown together with a mean, 95% confidence interval error bars, minimum, maximum, first quartile, median, and third quartile values for each sampling season.

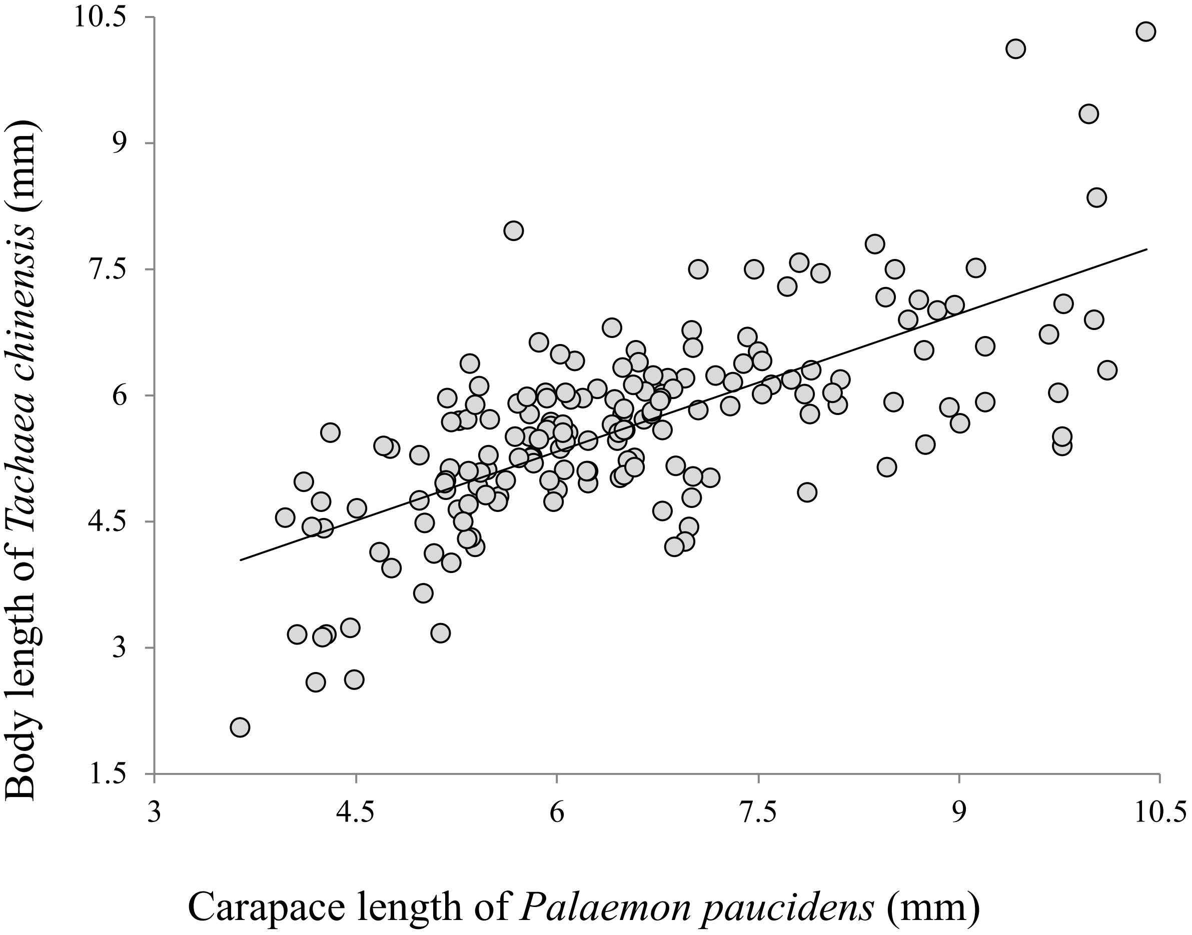


Figure 2.5: Relationship between carapace length (mm) of *Palaemon paucidens* and body length of *Tachaea chinensis* (mm). The linear regression equation and probability value is: ($y = 0.5478x + 2.0417$; $r^2 = 0.479$, $P < 0.01$, $n = 181$).

2.4 Discussion

2.4.1 Prevalence of *T. chinensis* on host shrimps

In Japan, *T. chinensis* is found on the lateral side of the carapace of atyids, palaemonids, and penaeids shrimps' families. While it was found infesting *Palaemon paucidens* in Honshu and shikoku regions, infestation on Caridina shrimps were obsereved in Okinawa Islands (Aoyagi 2014; Nagasawa et al. 2018). In China, the infested shrimp's species were to be entirely different. *T. chinensis* were found infesting on *Palaemon carinicauda* (Holthuis, 1950 and *Palaemon sinensis* (Sollaud, 1911) (Xu et al. 2019). The observed infestation pattern of *T. chinensis* is greatly determined by the density and distribution of the potential host shrimp in a particular habitat. This suggestion is supported by Ota (2019), who studies the abundance of this isopod in nature. The author found that at sampling sites were *Neocaridina* spp. is more dominant compared to *Paratya improvisa* and *P. paucidens*, *T. chinensis* isopods were found mainly infesting the dominant *Neocaridina* spp. host shrimp.

Likewise, in this study the isopods were found on the leading species *P. paucidens* in sampling months from August to June. The dominance for *P. paucidens* shrimps was 76.1 % while only 23.9 % dominance were recorded for *Neocaridina* spp. Despite the observed all year-round appearance of *Neocaridina* spp. shrimps at the same sampling sites, records on the infestations by *T. chinensis* were only observed from August to November (Mean prevalence 6.4 %). The infestation pattern is likely linked to the increased abundance of this host shrimp. As Ota (2019) indicated, that *Neocaridina* spp. showed an increased in the abundance reaching to its maximum peaks in these particular months. Therefore, in agreement with the suggestion made by Nagasawa et al. (2018), *T. chinensis* could infest on the abundant *P. paucidens* at some localities, while it might utilize *Neocaridina* spp. shrimps as an additional potential host at sampling sites where *P. paucidens* is less abundant.

2.4.2 Growth of *T. chinensis*

Tachaea chinensis were found attaching on the host shrimps in all months except for June and July in Fukui and Tokyo (Nakachi et al. 2010; Takahashi 2015). Moreover, Ota (2019) indicated that juvenile stages of this isopod were observed to attach on the host shrimps between autumn and spring. While it detaches from the host for reproduction under stones and dead plants at the bottom of the lake. Similar findings were obtained in this study in regards the periodic infestation pattern by juvenile isopods in shrimps. From August to September, the body length of *Tachaea chinensis* showed a rapid increase followed by an almost unvarying period between October to March, before it rapidly increased again in May and June of the following year. Indicating that the growth of these isopods is greatly influenced by water temperatures above 20°C in lake Biwa. Similarly, was concluded by Nakachi et al. (2010), in which the feeding behaviour of *T. chinensis* was suggested to decline during winter months.

2.4.3 Parasite-host size relationship

The current study demonstrated that, the body length of *T. chinensis* were found ranging between 2.0 – 10.3 mm in the host shrimp *P. paucidens*, while this range in body length considerably decreased on the host shrimp *Neocaridina* spp. (2.0 – 5.8 mm). Suggesting that larger isopods (6.0–10.3 mm) were found abundantly selecting *P. paucidens* as preferred host shrimp. These findings are consistent with other reports studying the body lengths of parasites on host shrimps. Ota (2019) found that greater body length scores were obtained from isopods retrieved from *P. paucidens* (1.5–7.8 mm) compared to *Neocaridina* spp. (1.5–5.8 mm). The same author also indicated that newly hatched isopods (Manca stage) are obligated to find a host as soon as they leave the brood pouch, suggesting Manca stage isopods may spend a considerably shorter periods of time as a free living ectoparasites. In this study, there is no significant difference to be found between the body length of parasites on the host shrimps *P. paucidens* (mean ± SD 3.4 ± 0.9 mm) and *Neocaridina* spp. (mean ± SD 2.9 ± 1.0 mm) in

August 2019. In the contrary, the isopods body length retrieved from the two host shrimps were found different in September of the same year. The two hast shrimps showed no difference in regards to the carapace length in August 2019. Interestingly however, *P. paucidens* were found noticeably larger compared to *Neocaridina* spp in September of the same year. Although the mean carapace of both shrimps did not exceed 6 mm in August – September, it rapidly surpassed 6 mm in carapace length for *P. paucidens* host shrimps from October to June. Hence, *T. chinensis* could infest both *P. paucidens* and *Neocaridina* spp. soon after hatching. The host specificity of the isopods was found different at different life stages; small *T. chinensis* (< 6 mm) were found infesting both shrimps (*P. paucidens* (37.5–96.2%) and *Neocaridina* spp. (3.8–62.5%)), but larger isopods showed an exclusive selectivity towards *P. paucidens* host shrimp.

In field and laboratory conditions, incidence of ectoparasites dislodgment often occurs in the middle of host's molt process. Although some of the isopods were successful in evading cast-off with the exoskeleton via a temporary change of attachment position (Xu et al. 2021). Therefore, host switching occasions by the parasite remains plausible during these unavoidable circumstances.

In this study large isopods (> 6 mm), appeared to prefer host shrimps whom carapace length is large enough to sustain infection. Which is supported by Ota (2019), who indicated that *Neocaridina* spp. carapace length might become a limiting factor of choice for larger isopods, as it might not provide enough space for attachment. The similarity in the body length to carapace length ratio of 0.8–0.9 between the isopod and host shrimp indicated that, the isopod is selecting host shrimp that are slightly larger than their own body size. Although the infestation by early-life-stage individuals of *T. chinensis* was observed on both shrimp species, *P. paucidens* appeared to be the principal host for individuals larger than 6 mm in body length.

Therefore, demonstrating a size selective behaviour that would grant *T. chinensis* safe attachment and prevents from dislodgment in nature.

Chapter 3

Host size selection in the ectoparasite *Tachaea chinensis* (Isopoda: Corallanidae) under laboratory condition

3.1 Introduction

Symbiosis is best described as a wide range of heterospecific associations characterized by a variety of adaptive interactions. Such interactions could be further divided into different categories based on its nature (Castro 1988). Usually separated into positive (e.g., mutualism) and negative (e.g., parasitism) interactions (Rosenberg and Zilber-Rosenberg 2011). Symbiosis, primarily parasitism, must not be confused with prey-predator interactions, as the latter involves a momentary interaction and the rapid distraction of one animal over the other (Castro 1988).

The order Isopoda (Crustacea), in particular, includes both free-living and/or parasitic species, many of which are found in the freshwater, marine waters, and even terrestrial environments. They can be found in association with fish, crustaceans and, in rare cases, echinoderms (Poulin 1995). The degree of the symbiotic mode of life varies greatly among parasitic isopods. Some are permanent (e.g., Cymothoids and Gnathiids) while others are temporary or accidental parasites (e.g., Aegids, Cirolanids and Corallanids) (Bunkley-williams and Williams 1998). However, in either modes of life, locating suitable habitats/ prey requires the use of sophisticated sensory cues by the parasites.

Beside the ability of fast recognition of suitable hosts, the size of the host could play a vital role in the distribution of a given parasite. Ota (2019), concluded that the size of *Neocaridina* spp. host shrimp displayed a limiting factor for larger isopods attachments, as larger isopods were found dominating the relatively large *Paleamon paucidens*. Likewise, the body size of bopyrid isopods was found to increase with the body size of their host (Pralon et al. 2018). The same study also indicated that, large sized isopods were found exclusively on larger host specimens. In contrast, Nagel and Grutter (2007), found out that the size was not a significant predictor of choice for the gnathiid isopods preference for host fish. While, in other study, it is concluded that such positive relationship between the parasite and host wasn't established between juvenile isopods infecting fish species, therefore, the basis behind isopods preference (e.g., type, size, behavior, life stage, etc.) for one host over another is still unclear (Nagel and Grutter 2007). Moreover, size selection is different at different life stages of the parasitic isopod. Which implies that they only grow to a level that provide protection from accidental dislodgment during molting (e.g., crustaceans) or swim drag (e.g., fish) caused by the host (Poulin 1995). Therefore, it can be suggested that smaller individuals could choose a larger space that would not restrict their growth in the future (Welicky et al. 2019).

The superorder Flabellifera includes the family Corallanidae, which is a closely related and superficially similar family to the Cirolanidae, Aegidae and Cymothoidae families (Bruce et al. 1982). Species of this family characterized by the presence of both free living and parasitic isopods that are distributed in freshwater, brackish water as well as the marine environment (Delaney 1989). The genus *Tachaea* contains six species commonly known as temporary parasites restricted to the Indo-West pacific region. They are often seen infesting wide range of freshwater shrimps, whereby only one species found in the marine habitat (Delaney 1989). The wide range of potential hosts may rise the question about the initial search and attachment for suitable host in these ectoparasites.

Tachaea chinensis isopods are usually found on the carapace of different host shrimps in China, Japan, Vietnam, Thailand and Malaysia (Xu et al. 2019; Nagasawa et al. 2018; Ota 2019). They are known to affect the immune and the antioxidant systems of the associated host shrimps (Li et al. 2020). The lesions left by the sharp mouthpart structures of the isopods are also believed to attract secondary infections such as *Vibrio* sp. (Ueki et al. 1988; Li et al. 2021). Displaying a threat to ecologically important and commercially viable species of freshwater shrimps.

Given these harmful potentials, little research has been devoted to the mechanism behind the initial search and/or host preference in these isopods.

During unavoidable circumstances, incidence of parasite detachments usually occurs in nature. However, it remains unclear whether the re-establishment is made on the basis of suitable size, suitable host species or both. Hence, it is important to explore the basis behind host selection in these parasites in order to fully understand its preference across potential hosts and thus the development of better mitigation measures in the future. In this study, we investigate the size selection behavior of the isopod *T. chinensis* across different host species in laboratory conditions. *P. sinensis* is a non-native freshwater shrimps, introduced from China via ornamental pet trade and as fishing baits (Saito 2017, 2018; Saito et al. 2019; Ogasawara et al. 2021; Imai et al. 2021). Hypothetically, providing an additional host shrimp for *T. chinensis* infestation and distribution. Therefore, the following question will be addressed in this study: Does *T. chinensis* prefers a certain host size regardless of host species?

3.2 Materials and methods

3.2.1 Host shrimps and parasites collection

Host shrimps and *T. chinensis* isopods were collected from water ways and rice fields irrigation canals in Shimane prefecture and Okayama prefecture - Japan during March 2022. *T. chinensis* in this study was exclusively retrieved from the dominant host *P. paucidens*. Experimental shrimps; *P. paucidens* and *Palaemon sinensis* was collected from the sampling sites while *Neocaridina* spp., were reared and maintained earlier in the laboratory. Host shrimps and isopods were scooped using hand nets (2.5 mm mesh size, 35 x 35 cm frame), lowered around the submerged vegetation and water-ways walls in 10 – 60 cm depth range. Specimens were placed in coolers fitted with portable aerators and transported to the laboratory of Aquatic Ecology at Hiroshima university.

In the laboratory, host shrimps were sorted and placed in separate aquaria (58.5 cm x 15.5 cm x 21.5 cm) fitted with a circulating filtration systems and aerations, to avoid potential cannibalistic behavior. Water temperature in each aquarium was maintained at 24 ± 1°C and received a 12 h light and 12 h dark cycles using an automated artificial lighting. Host shrimps were fed daily, once ad libitum, with commercial feeds (Hikari Ronchu discs, 1.3 – 1.5 mm, KYORIN – Japan). *T. chinensis* isopods were gently detached from the host shrimps and placed in 500 ml plastic containers supplied with aeration, without food, and used in the treatments within 10 days from sampling. Starvation of the isopod was limited to 24 hours prior experimenting; whereby longer durations have been shown to greatly affect the activity levels of the organism (Xu et al., 2021).

The carapace length of host shrimps and the body length of *T. chinensis* were measured to the nearest 0.1 cm. The carapace length of the host shrimps was measured from the orbital edge to the posterior margin. While the body length of the isopod was measured from the boarder of the head to the end of the uropods. All shrimps and parasites were allowed to acclimated for at least 48 h before the experiment.

3.2.2 Laboratory selection experiments

Size selection experiments were conducted using small plexiglass tanks (17.5 cm x 8.4 cm x 6.5 cm) divided by two plastic barriers that allowed the isopods to pass freely but prevented the passage of the two experimental shrimps (Figure 3.1). A total of 6 treatments were conducted involving different size combinations of host shrimps; *P. paucidens, P. sinensis* and *Neocaridina* spp. (Table 3.1). The purpose of treatments 1 – 4 is to investigate the preference of *T. chinensis* in the presence of different sizes of the same host species. While treatment 5 were designed to test whether *T. chinensis* demonstrate host preference and/or size selection behavior regardless of the host species. The ratio between the body length of *T. chinensis* to the carapace length of host shrimps was used to standardize the size of the experimental animals in all treatments. The carapace length for each shrimp was set to be small (S) if the ratio between the body length of the isopod to carapace length of host shrimp is 1.2 – 2.0. Whereas the medium (M) and large (L) ratios were set to be between (0.7 – 1.0) and (0.2 – 0.5), respectively.

In each treatment, the experimental tank was washed and filled with dechlorinated tap water, to avoid chemical cues that might alter the selection behavior of the parasite. Subsequently, the shrimps were placed at the opposite ends of the experimental tank (8.6 cm apart), *T. chinensis* was introduced at the middle of the tank using a transparent glass container and allowed to acclimate for 20 minutes before release. Individual isopods were tested once, in which different isopods and host shrimps were used in each treatment (10 isopods per treatment). In order to avoid a possible escaping behavior instead of an actual preference, the experiment was run for 18 h after overnight exposure. The selection made by the isopod was recorded after 3 and 18 h. The isopod was considered to select a shrimp if an attachment on the carapace of the chosen shrimp was observed. If *T. chinensis* was found un-attached to any of the provided host option after 18 h the results were considered null. To avoid any acquired behavior, different host shrimps/isopods were used for each treatment/trial.

The purpose of treatment 6 is to evaluate the size selection pattern of newly hatched (1 day after hatch) *T. chinensis* when exposed to large and small host shrimp of *Neocaridina* spp. Egg-bearing isopods were obtained from Okayama prefecture in June 2022. All parasites were allowed to acclimate along with the associated host shrimps in the laboratory until hatching. Upon hatching, manca isopods were collected using small pipette (3 ml) and transferred to the experimental tanks. A total of 10 manca stage isopods were tested for its preference in this treatment.

3.2.3 Statistical analysis

The binomial test of significance was used to compare the final position of the isopod against the theoretical probability of 50%. In which the selection of host option A or host option B, no selection (Null) and/or predation incidences had an equal probability of occurrence. To understand whether the performance of *T. chinensis* changes during the presence of the natural host, the difference between the infested proportions (e.g., positive outcome) and (Null) from the two time points (3h and 18 h) were validated for each treatment using fisher exact test followed by a pairwise comparison with Bonferroni correction. To assess the effect of host shrimp size on the selection made by the isopod, a logistic regression analysis was used. All computation were done using R statistical software (Version 4.0.3, R foundation for statistical computing, Vienna, Austria).

3.3 Results

Upon release into the tank, the majority of isopods were found swimming towards either tank's zones. While others were found initially hiding under the tank's gates before finally approaching the host shrimps and perform several attachments attempts. Isopods attachment process starts when the isopod strongly clings to any of the shrimps' appendages, a process usually a combined with host vigorous movements, until it finally settles on either side of the carapace. A total of 70 individuals of *T. chinensis* were used in this study. Almost all tested isopods demonstrated the attachment behavior, in which 87.1% had attached to the carapace of the provided host subjects with only 10% and 2.9% showed null and predation results respectively. Host switching between 3- and 18-hours' time points was recorded in only 4 isopods switching between the two provided host options in the tank during the study. The results from the Fisher exact test and the logistic regression analysis were insignificant ($P > 0.05$), however, varying results from the binomial test of significance were obtained in each of the treatments.

3.3.1 Treatment 1

In treatment 1, *T. chinensis* selected medium sized *P. paucidens* more frequently than the small host (60%; binomial test, $P < 0.05$) (Figure 3.2).

3.3.2 Treatment 2

The selection activity of *T. chinensis* in the presence of large size and medium sized *P. paucidens* demonstrated a significant pattern (binomial test, $P > 0.05$). Only one isopod switched from the large host at the 3 hours timepoint to the medium host after 18 hours. *T. chinensis* selected the medium sized *P. paucidens* (60%) more than the larger host option (30%). Whereby, a single isopod was consumed (e.g., predation) by the large host at the 3 hours timepoint (Figure 3.3).

3.3.3 Treatment 3

The selection behavior of *T. chinensis* was found non-significant in the presence of different sizes of *P. sinensis* (Figure 3.4). Only 70% showed an attachment on either host options, while 30% of the isopods did not select any of the provided hosts sizes at the end of the treatment (binomial test, $P > 0.05$).

3.3.4 Treatment 4

In the treatment involving size combinations of *Neocaridina* spp. host shrimp, *T. chinensis* showed a significant selection behavior (80%) towards medium host shrimps (Figure 3.5). Only 20% selected the small *Neocaridina* spp. (binomial test, $P < 0.001$). In which three isopods switched hosts from the small host at the 3 hours timepoint to the medium host after 18 hours.

3.3.5 Treatments 5

In this treatment, size combinations of different host species were provided for *T. chinensis* (e.g., *P. paucidens* and *Neocaridina* spp.). The infestation rate was 100% in both trials, with *T. chinensis* selecting either host/size object provided. When subjected with medium *P. paucidens* and small *Neocaridina* spp., 9 out of 10 of *T. chinensis* selected the medium *P. paucidens* (binomial Z test, $P < 0.001$). In the presence of small *P. paucidens* and medium *Neocaridina* spp., a significant proportion of *T. chinensis* were found selecting the small *P. paucidens* (binomial test, $P < 0.05$), although infestation on the medium *Neocaridina* spp., increased to 40% (Figure 3.6).

3.3.6 Treatment 6

Manca staged *T. chinensis* did not show an obvious selection preference between large and small sized *Neocaridina* spp. Instead, infestation rate was 50%, in which the remaining 50% did not successfully attached and/or consumed by the host shrimp (Figure 3.7). An individual isopod was found consumed by the large *Neocaridina* spp. host shrimp during this treatment.

Table 3.1: Experimental treatments for assessing the size selection behavior of *Tachaea chinensis*.

Treatments	*Tachaea chinensis* average body length (mm)	Host[a]		Host choice A[b]	Host choice B	n
		Zone A	Zone B			
1	6.3	*Paleamon paucidens*	*Paleamon paucidens*	Medium (0.82)	Small (1.36)	10
2	4.7	*Paleamon paucidens*	*Paleamon paucidens*	Large (0.49)	Medium (0.90)	10
3	7.1	*Paleamon sinensis*	*Paleamon sinensis*	Medium (0.84)	Small (1.33)	10
4	5.7	*Neocaridina* spp.	*Neocaridina* spp.	Medium (0.96)	Small (1.90)	10
5	5.6	*Paleamon paucidens*	*Neocaridina* spp.	Medium (0.84)	Small (1.55)	10
	5.0	*Paleamon paucidens*	*Neocaridina* spp.	Small (1.21)	Medium (0.83)	10
6	1.9	*Neocaridina* spp.	*Neocaridina* spp.	Large (0.37)	Medium (1.0)	10

[a] A and B indicates the two opposite ends of the experimental tank.

[b] Mean ratios between Tachaea chinensis body length and hosts' carapace length; small (1.2 – 2.0), medium (0.7 – 1.0) and large (0.2 – 0.5)

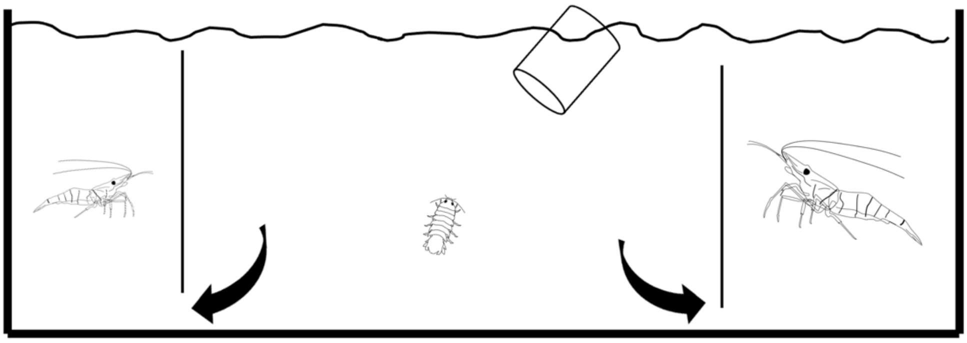


Figure 3.1: Schematic representation of the experimental system used to test size selection behavior of the isopods.

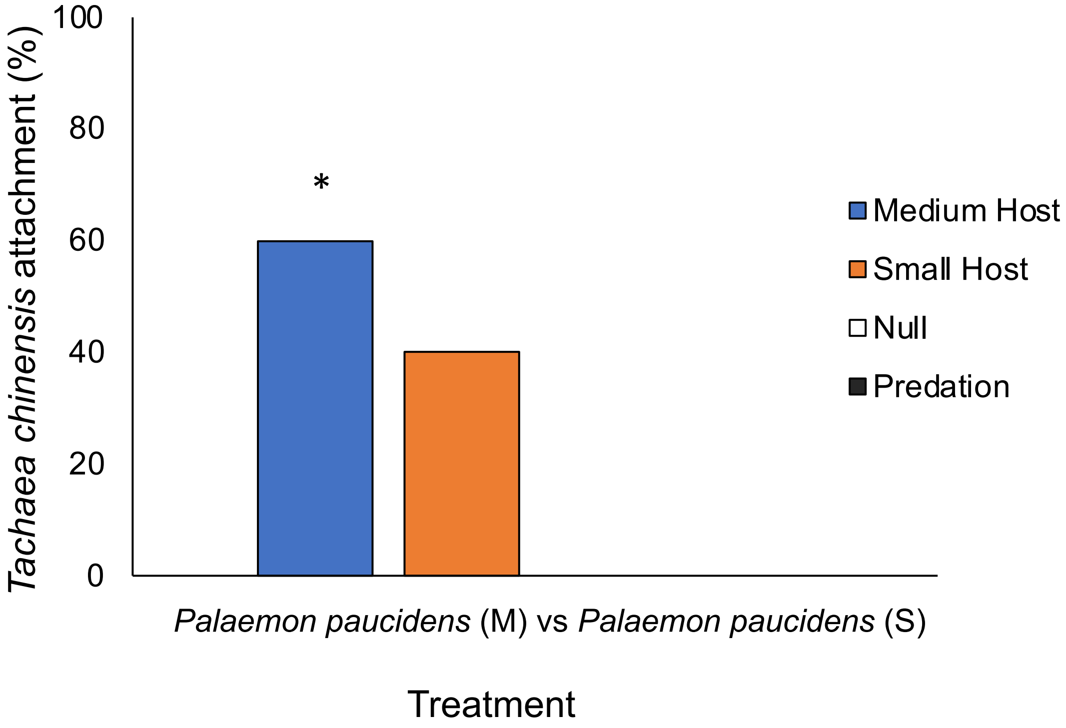


Figure 3.2: Selection percentage of *Tachaea chinensis* (n = 10) for treatments involving medium and small *Palaemon paucidens*. M = medium and S = small; *: $P < 0.05$ (Binomial test of significance).

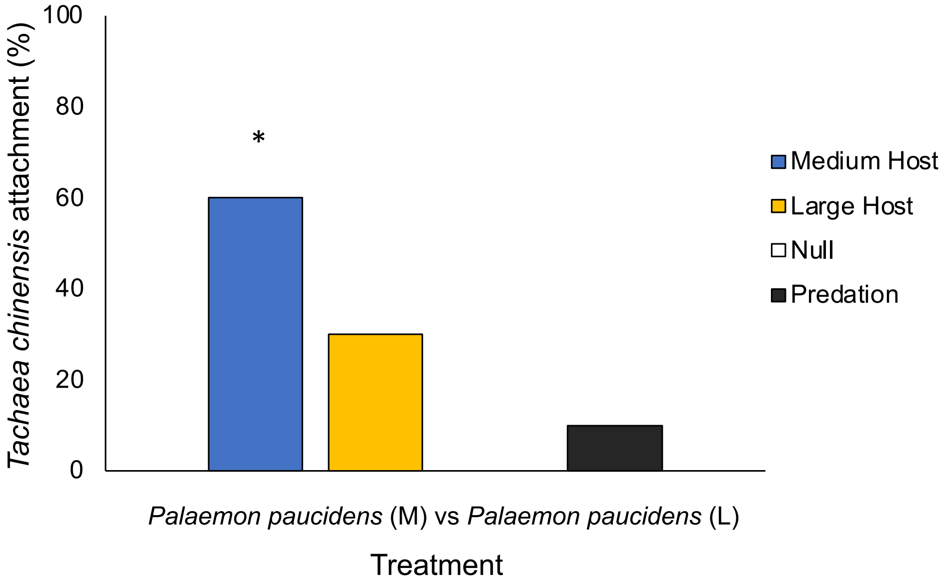


Figure 3.3: Selection percentage of *Tachaea chinensis* (n = 10) for treatments involving large and medium *Palaemon paucidens*. L = large and M = medium; *: $P < 0.05$ (Binomial test of significance).

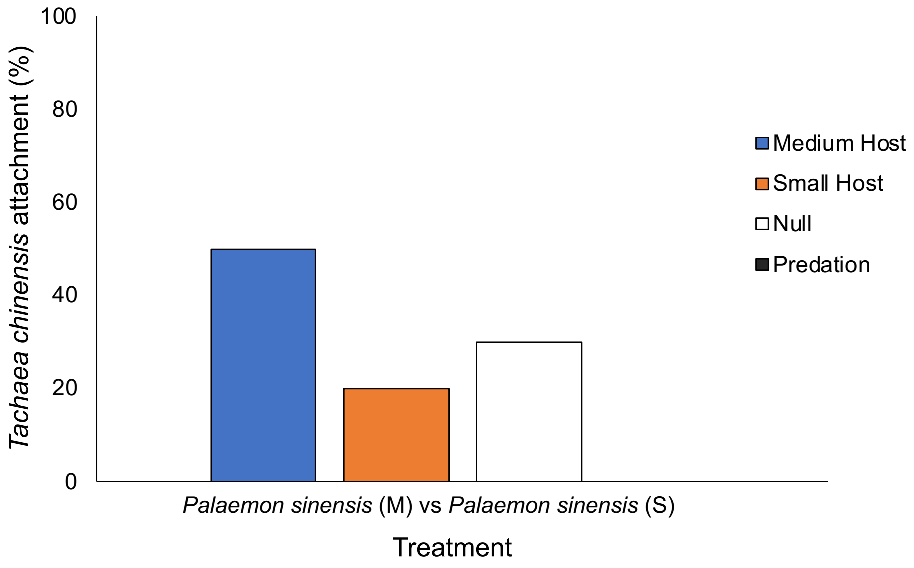


Figure 3.4: Selection percentage of *Tachaea chinensis* (n = 10) for treatments involving medium and small *Palaemon sinensis*. M = medium and S = small.

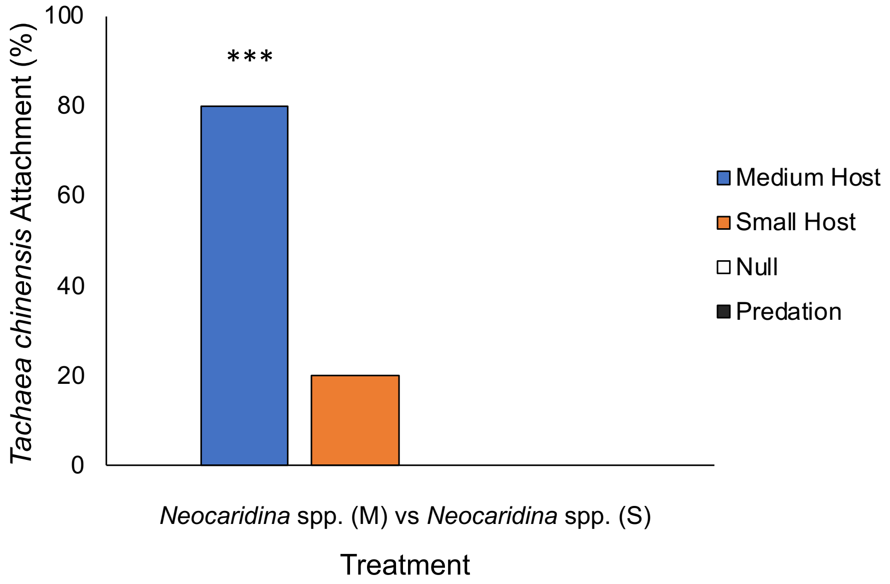


Figure 3.5: Selection percentage of *Tachaea chinensis* (n = 10) for treatments involving medium and small *Neocaridina* spp. M = medium and S = small, ***: $P < 0.001$ (Binomial test of significance).

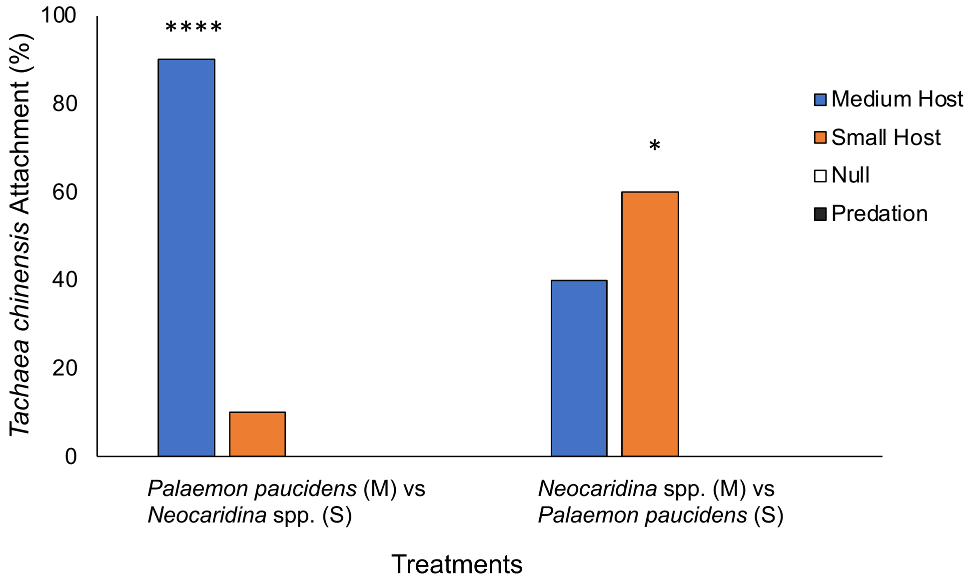


Figure 3.6: Selection percentage of *Tachaea chinensis* (n = 10) for treatments involving a mixed pairs of *Palaemon paucidens* and *Neocaridina* spp. with different sizes. M = medium and S = small. *: $P < 0.05$ and ****: $P < 0.0001$ (Binomial test of significance).

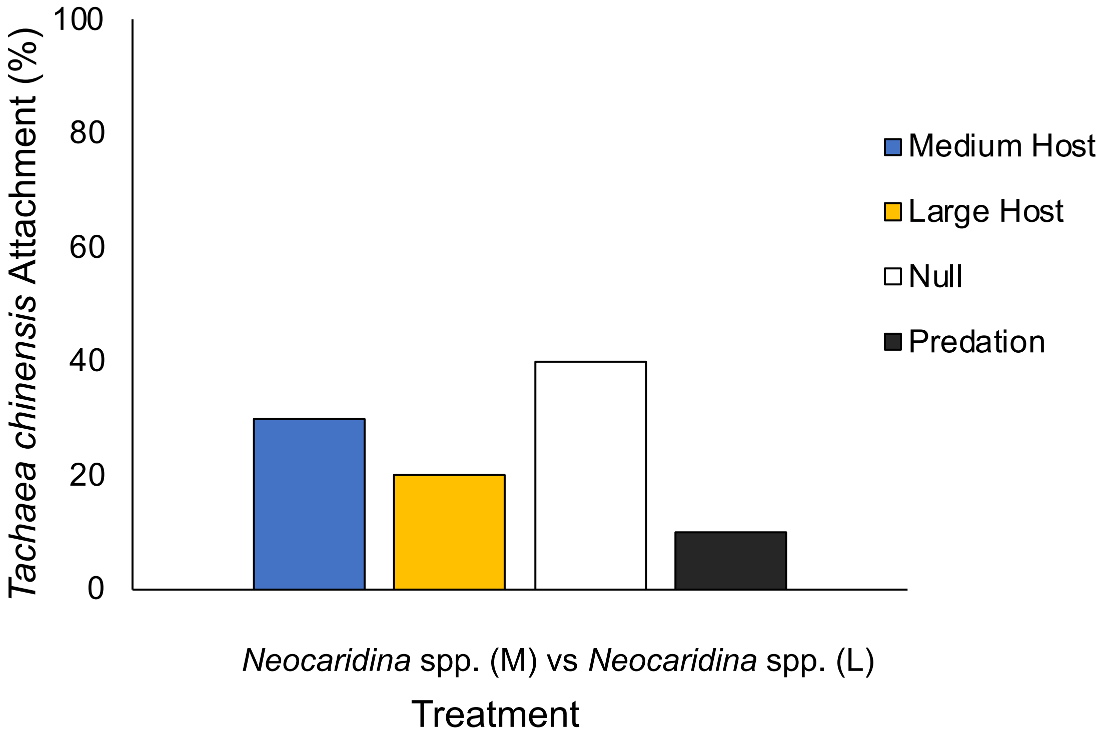


Figure 3.7: Selection percentage of Manca stage *Tachaea chinensis* (n = 10) in treatments involving *Neocaridina* spp. pairs of different sizes. M = medium and L = large.

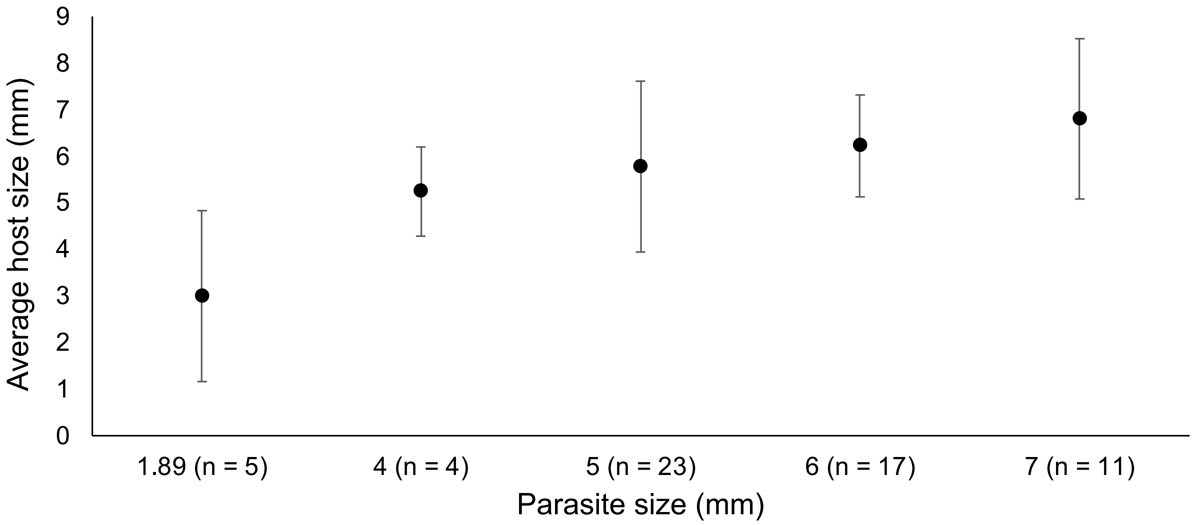


Figure 3.8: Selected host size by *Tachaea chinensis* for each size class in all treatments. Data are means ± standard deviations.

3.4 Discussion

Ectoparasitic crustaceans rarely stay on the same host indefinitely; instead, some are forced to change hosts when threatened or in the search for a new host at the incidence of host death. However, such process is usually limited by the low number of available hosts and/or usually governed by environmental and biological factors (Boxshall and Hayes 2019). Once the infection is initiated, the parasite undertakes several adaptations to cope with its new host (Baillie et al. 2019). Hence, how parasites locate suitable hosts and the complex sensory recognition system is a determinant factor ensuring the survival of these parasitic organisms (Tokaji et al. 2014; West and Brooks 2018).

The results from these experiments demonstrated that *T. chinensis* were able to recognize medium host shrimps in pairwise selection treatments. Higher proportions of isopods were found attaching on shrimps having medium carapace length regardless of host species. Observational records from the wild, also confirmed that the isopods *T. chinensis* commonly seen associated with hosts shrimps that's are slightly larger than their own body length (Nagasawa et al. 2018; Khalfan et al. 2022). In this study, the isopod was found swimming directly to items placed in the tanks such as the provided host options, indicating the presence of strong visual abilities and/or other sensory capabilities. However, stimulants such as visual, chemical and tactile were all presents in the treatments making it difficult to pinpoint which of the sensory mechanisms was the strongest. Further research is needed to solely investigate each of these sensory modulations.

Ectoparasite isopods tend to only grow to a level that provide protection from accidental dislodgment during molting (e.g., crustaceans) or swim drag (e.g., fish) caused by the host (Poulin 1995). Therefore, smaller host shrimps may provide less favorable space for large isopods to safely attach (Ota 2019). The effect of host size on *T. chinensis* selection was more prominent when exposed to medium *P. paucidens* vs small *Neocaridina* spp., in which the majority were found attaching on the medium host. However, when exposed to the medium *Neocaridina* spp. vs small *P. paucidens* treatment, the isopod tended to significantly select the smaller *P. paucidens*. This result could be explained by the influence of the original source host, as *T. chinensis* was solely retrieved from *P. paucidens* host shrimps. This host shrimp was known to be the preferred host for the isopod in Honshu and Shikoku islands in Japan (Nagasawa et al. 2018).

The occurrence of *T. chinensis* might be influenced by the abundance of host shrimps at a given geographical site (Nagasawa et al. 2018). However, the number of host shrimps available for the isopods were held equal in all trials. Therefore, species abundance was hardly the only determinant factor for isopods selection pattern. Although the reason behind the null results of *T. chinensis* in treatments involving *P. sinensis* is unknown, observational records suggests that the exotic *P. sinensis* might be more active and thus could easily escape infestation compared to *P. paucidens* and *Neocaridina* spp.. Incidence of isopods host switching was observed between 3 h and 18 h time points in this study. It is also possible that, this behavior in few isopods is temporary and beyond the experiment time scope (18 h).

Size selection could be different at different life stage of the parasitic isopod. Therefore, smaller individuals might select a larger space that would not restrict their growth in the future (Welicky et al. 2019). However, results from manca stage treatment demonstrated otherwise. Manca staged isopods were seen attached randomly to both large sized and small size *Neocaridina* spp. host shrimps (Figure 3.7). Similar finding was observed by Xu et al. (2021), where manca stage isopods showed low host specificity in laboratory condition.

The size selection behavior of the isopod could also be governed by the apparent risk of predation by the chosen host (Ota 2013). In which larger host option could resemble a greater probability of predation. Incidences of host predation on manca stage *T. chinensis* were detected in other study (Xu et al. 2021) as well as during the current study. Similarly, Penfold et al. (2008) studied the predation and micropredation by gnathiid isopods on coral reef fishes and found that while fishes from either size were able to prey on the isopods, larger hosts consumed more isopods compared to smaller hosts.

To conclude, our results suggest that *T. chinensis* likely to show size specificity according to the developmental stage. The parasite responded well in the presence of different host sizes (attachment percentage: > 80%). Moreover, when provided with pairs of different host's carapace length, *T. chinensis* may select hosts shrimps that are slightly larger than their own body length. Moreover, upon analyzing the size of the tested isopods against the selected host carapace length, the selected carapace length tended to increase with the increase of parasites body length (Figure 3.8). Additionally, the current findings suggest that when large differences, in carapace length, between host pairs exist, *T. chinensis* express more size selection behavior regardless of the host species, which ensures suitable space for attachment and growth. This result complies with Ota (2019) and Khalfan et al. (2022) who indicated that the differences in prevalence of *T. chinensis* may be linked to the ontogeny speed and size at maturity of *T. chinensis* common host shrimps in the natural habitat. The current study demonstrated the size selection behavioral of *T. chinensis* on three common host shrimps, therefore, further studies including other host shrimps are needed to understand their host selection and utilization mechanisms.

Chapter 4

Host species selection of *Tachaea chinensis* (Isopoda: Corallanidae) and host-parasite predation under laboratory conditions

4.1 Introduction

The infraorder Caridea contains a group of shrimps known as the Palaemonids shrimps. These shrimps can be found in various habitats including marine, brackish and freshwater habitats (Grave et al. 2007). Together with other organisms, these freshwater shrimps have shown to play a vital role in the establishment and maintenance of freshwater habitats diversity and communities' composition (March et al. 2002). However, as a results of their extensive use in the ornamental industries and as baits in recreational fishing, many of these shrimps end up relocated and/or introduced to new habitats (Niwa 2010; Saito et al. 2011; Saito et al. 2019). Alongside the imposed anthropogonic pressure on these shrimps via trade, many are often seen infected by different types of parasites (e.g., Bopyrids and Corallanids). Through attachment on the carapace of the host shrimps, these parasites are able feed on the hemolymph and develop diverse effects on its associated hosts. Such effects include; increase energy burden, reproductive failures and even pave the way for various pathogenic infections (Bass and Weis 1999; Yu et al. 2021). A good example of these parasites includes the Corallanid isopods. This family of isopods is a home to both free living and permanent-parasites infecting fish, turtles, mysids and shrimps (Guzman et al. 1988). In addition, Corallanidae includes the genus *Tachaea,* which are a group of parasites are known as temporary ectoparasites infecting freshwater shrimps, sponges and fish (Delaney 1989; Stebbing 1907; Sahoo et al. 2022).

Typically, the infestation pattern and prevalence of these groups of parasites are suggested to be governed by the density of a given host species at a given location (Thompson 1995; Ota 2019). In Japan, for example, the isopod *Tachaea chinensis* has been found on different host shrimps, depending on the abundance of that shrimp (Ota 2019; Nagasawa et al. 2018).

Whereas, in China, the same parasite often infests on different host shrimp's species (e.g., *Palaemon sinensis*) (Xu et al. 2019). Despite their regular occurrence, research on these isopods has been limited to the taxonomy and distribution and pathology, in which little attention has been given to the host-parasite interactions in regards to host preference. The host-parasite interactions are usually controlled by complex environmental and/or biological factors, whereby the mechanism and the basis behind the isopods host choice remain unclear (Leung and Poulin 2008; Nagel and Grutter 2007). However, host size and the associated available space are likely to resemble a potential factor of choice for these parasites. A previous study observed that large isopod *Tachaea chinensis* demonstrated a size selection behavior towards the large *P. paucidens* host shrimps. The length ratios between host carapace and the body of parasite were constant 0.8–0.9. Indicating that the parasitic isopod repeatedly found infesting host shrimps that are slight bigger than their own body size (Khalfan et al. 2022). Other reports also suggested that, the relatively small *Neocaridina spp.* host shrimps might not provide enough space for larger isopods attachments (Ota 2019). Yet, small host shrimps could offer a less harmful alternative for the parasite to infest. As the latter may lack the strength and the experienced defense system. Therefore, resulting in less probability of predation. For example, Xu et al. (2021) found that host shrimps were frequently observed preying on the newly hatched manca staged *Tachaea chinensis* under laboratory conditions. Similarly in different parasitic species, fish of various sizes were found consuming the isopods, although larger hosts ate relatively larger amounts (Penfold et al. 2008). Therefore, larger hosts could demonstrate a higher chance of predation compared to smaller host. Nonetheless, to date the isopods' ability to demonstrate a clear pattern of host specificity or the role of predation on isopods selection and population dynamics remained unclear.

In this study we aim to investigate the host selection behavior of *Tachaea chinensis* isopods using pairwise choice experiments. In laboratory condition, we examined the isopods preference behavior in a series of uncommon hosts, shelter place and no choice scenarios. Moreover, we shed light on the susceptibility of host shrimp's predation on the *Tachaea chinensis* isopod.

4.2 Materials and methods

4.2.1 Sampling of host shrimps and parasitic isopods

Tachaea chinensis (Isopoda: Corallanidae) and host shrimps were collected from ponds and water ways from Okayama prefecture (35°01'52.6"N 133°48'32.3"E) and Shimane prefecture (35°04'34.0"N 132°33'43.0"E), Japan, from August 2021 till January 2022. Host shrimps and parasites were scooped using hand nets (35×35 cm; mesh size, 2.5 mm; handle length, 240 cm) from water ways side-walls and submerged vegetations. Parasitized Host shrimps and individual parasites were both placed in water filled containers fitted with aeration and transported to the laboratory of Aquatic Ecology at Hiroshima university.

In the laboratory, host shrimps along with attached parasites were acclimated in different acrylic tanks (58.5 cm x 15.5 cm x 21.5 cm, 57.5 cm x 26.8 cm x 30 cm and 43 cm x 5 cm x 21 cm) according to species. Water temperature in the acclimation tanks were maintained at 24 ± 1°C receiving 12D:12L automated light cycle. Host shrimps were fed daily with a commercial feed (Hikari Ronchu discs, 1.3 ~ 1.5 mm, KYORIN – Japan). All treatments were conducted only on active shrimps and isopods after 1 week of acclimation.

4.2.2 Host species selection experiments

A total of 13 treatments were conducted, each of which was repeated 10 times using different hosts and isopods (Table 4.1). Isopods were retrieved from a single source host *Paleamon paucidens*, in order to avoid choice behavior influenced by the difference in source host.

The treatments included the use of: un-common hosts, no choice and food vs shelter treatments. Un-common hosts involved the host shrimp *Macrobrachium nipponense* and Goby fish (*Rhinogobius* sp.), the invasive Crayfish *Procambarus clarkii,* and an artificial *Palaemon paucidens* (Fishing lures, HENGJIA Network Technology Co., Ltd, China). These trials were design to compare the choice behavior of *Tachaea chinensis* in the presence of un-common host that occurs at similar environments. The remaining two groups of experiments (e.g., no choice and food vs shelter) were conducted to investigate isopods preference between food and shelter. The carapace length of shrimps and the body length of *Tachaea chinensis* were measured to the nearest 0.1 cm. Host shrimp's carapace length was measured from the orbital edge to the posterior margin. While the body length of the isopod was measured from the boarder of the head to the end of the uropods. Isopods average size ranged between (5.3 – 7.3 mm) and were either similar and/or smaller than the carapace length provided host options. They were starved for 24 hours prior to experimenting. All provided choice pairs were at similar carapace length (e.g., shrimps) and/or total length (e.g., fish).

Host preference experiments were conducted using small plexiglass tanks (17.5 cm x 8.4 cm x 6.5 cm) divided by two plastic barriers that allowed the isopods to pass freely but prevented the passage of the experimental shrimps (Figure 4.1). In each treatment, the experimental tank was washed and filled with priorly prepared tap water, to avoid chemical cues that might alter the choice selection behavior of the parasite. The choice objects were then placed interchangeably at the opposite ends of the experimental tank (8.6 cm apart). After that, *Tachaea chinensis* was introduced at the middle of the tank using a transparent glass container and allowed to acclimate for 20 minutes before release. In order to avoid a possible escape behavior instead of an actual preference, the experiment was run for 18 hours after overnight exposure. The host preference was recorded based on the zone in which the isopods were found at each time point (figure 4.1).

If *Tachaea chinensis* was found in zone (3), after 18 hours, the results were considered null. Control trials (n = 10), without any choice object, were conducted to determine the preference of the isopod to a certain direction/zone in the experimental tank. The results indicated no specific preference to either zone 1 and zone 2 when all zones are empty.

In addition, preliminary experiments to evaluate the possibility of host shrimp's predation on the isopods *Tachaea chinensis* were conducted using Three shrimps' species; *Procambarus clarkii, Macrobrachium nipponense* and *Paleamon paucidens*. We hypothesized that the larger the difference between host shrimps' size and *Tachaea chinensis* body size, the higher the probability of predation. Thus, in each treatment, a single large host shrimp was provided with two *Tachaea chinensis* in the same tank. In which the difference between host carapace length and isopods total length was set to be large enough to investigate our hypothesis. Predation experiments were conducted using small plexiglass tanks (17.5 cm x 8.4 cm x 6.5 cm) (Figure 4.1). In each treatment, the body size to carapace length ratios were provided to be between 0.2 – 0.5, in order to investigate predation behavior by the shrimp's species. The number of *Tachaea chinensis* consumed by each host shrimp was then recorded at each time points (1, 2, 3, 6, 12, 24 hours).

4.2.3 Statistical analysis

The binomial test of significance was used to compare the final position of the isopod against the theoretical probability of 50 %. Whereby, recorded results (e.g., host option A, host option B, no selection (Null) and/or predation) had an equal probability of occurrence. Fisher exact test was used to investigate the relationship between host species and amount of *Tachaea chinensis* consumed in the predation experiments. The computation was done using R statistical software (Version 4.0.3, R foundation for statistical computing, Vienna, Austria).

4.3 Results

4.3.1 Un-common hosts

This treatment was design to investigate the host selection behavior of *Tachaea chinensis* when subjected to a choice between the source host and less common host species found in the wild. The results indicates that almost all of the isopods were found significantly associated with the common host shrimps *Palaemon paucidens* compared to *Rhinogobius* sp., *Macrobrachium nipponense Procambarus clarkii,* and the artificial *Palaemon paucidens* (Figure 4.2). The selection proportion was 80 % for the host selection treatments involving *Rhinogobius* sp. and *Procambarus clarkii* ($P < 0.001$; binomial test of significance).

4.3.2 Food and shelter

The main purpose these treatments is to investigate the selection behavior of *Tachaea chinensis* in the presence of a choice between potential host vs shelter plants as well as when provided with a no choice scenario (e.g., potential host only in the experimental tanks). The results from the host shrimp's vs shelter treatments revealed that *Tachaea chinensis* significantly preferred the host shrimps *Palaemon paucidens* (100 %), *Palaemon sinensis* (90 %) and *Neocaridina* spp. (80%), over shelter plants *(*$P < 0.001$ and $P < 0.0001$; binomial test of significance) (Figure 4.3). *Tachaea chinensis* showed a significant selection behavior when provided with a single host choice *Palaemon paucidens* (80 %; $P < 0.01$; binomial test), *Neocaridina* spp. (100 %), *Macrobrachium nipponense* (60 %) and *Procambarus clarkii* (90 %). However, it did not show a significant selection behavior when provided with *Palaemon sinensis* and the artificial *Palaemon paucidens* (Figure 4.4).

4.3.3 Predation preliminary experiment

All of the three treatments showed incidence of host predation on *Tachaea chinensis*. In treatments involving *Procambarus clarkii* crayfish, 96.9 % of tested isopods were consumed by the provided shrimp. Similarly, *Macrobrachium nipponense* consumed 71.9 % of the tested isopod. To a lower extant 28.6 % were consumed in treatments involving *Palaemon paucidens* shrimps. There was a strong significant relationship between the number of isopods consumed and shrimp's species; *Procambarus clarkii vs Palaemon paucidens* (Fishers exact test, $P < 0.0001$), *Procambarus clarkii vs Macrobrachium nipponense* (Fishers exact test, $P < 0.05$), and *Macrobrachium nipponense vs Palaemon paucidens* (Fishers exact test, $P < 0.01$) (Figure 4.5 – 4.8).

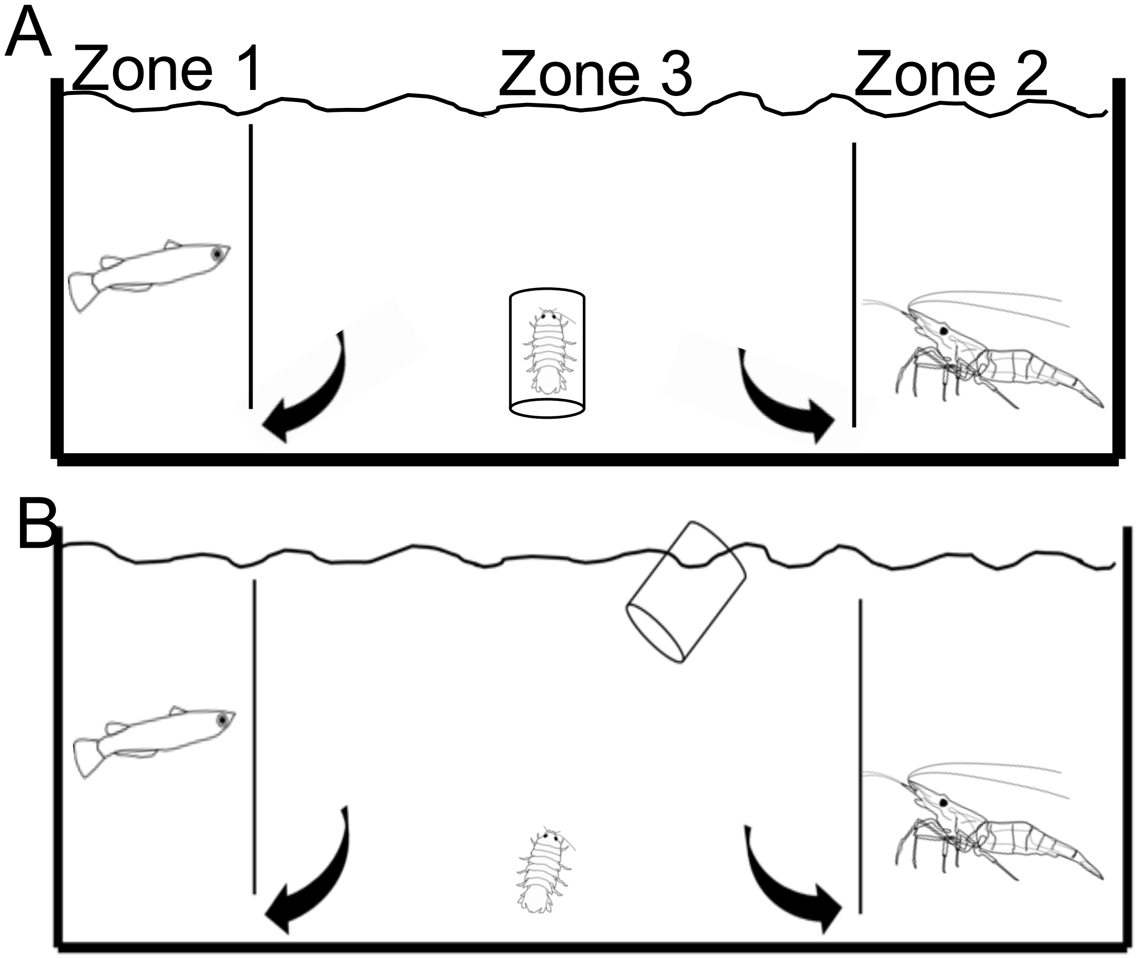


Figure 4.1: Schematic representation of the experimental system used to test size selection behavior of the isopods. A: *Tachaea chinensis* at 20-minute acclimation. B: *Tachaea chinensis* after release.

Table 4.1: Experimental treatments to investigate the host preference behavior of *Tachaea chinensis*. A and B indicates the two opposite ends of the experimental tank. Each treatment involves 10 replicates.

Experiment category	Treatments	Host		Number of Trials
		Zone A	Zone B	
Un-common host	1	*Palaemon paucidens*	*Rhinogobius* sp.	10
	2	*Palaemon paucidens*	*Macrobrachium nipponense*	10
	3	*Palaemon paucidens*	*Procambarus clarkii*	10
	4	*Palaemon paucidens*	Artificial *Palaemon paucidens*	10
Food vs shelter	5	*Palaemon paucidens*	*Egeria* sp.	10
	6	*Palaemon sinensis*	*Egeria* sp.	10
	7	*Neocaridina* spp.	*Egeria* sp.	10
No choice	8	*Palaemon paucidens*	Control	10
	9	*Palaemon sinensis*	Control	10
	10	*Neocaridina* spp.	Control	10
	11	*Macrobrachium nipponense*	Control	10
	12	*Procambarus clarkii*	Control	10
	13	Artificial *Palaemon paucidens*	Control	10

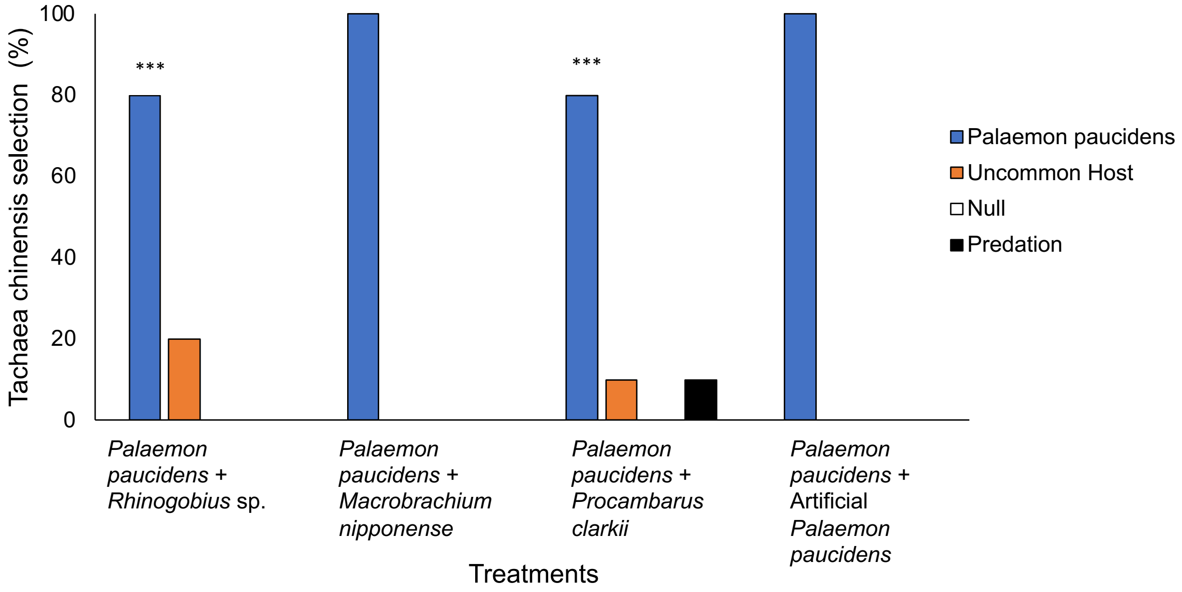


Figure 4.2: Selection percentage of *Tachaea chinensis* in the un-common host selection experiment. A sample size of 10 were used for each treatment; **: $P < 0.01$ (Binomial of significance).

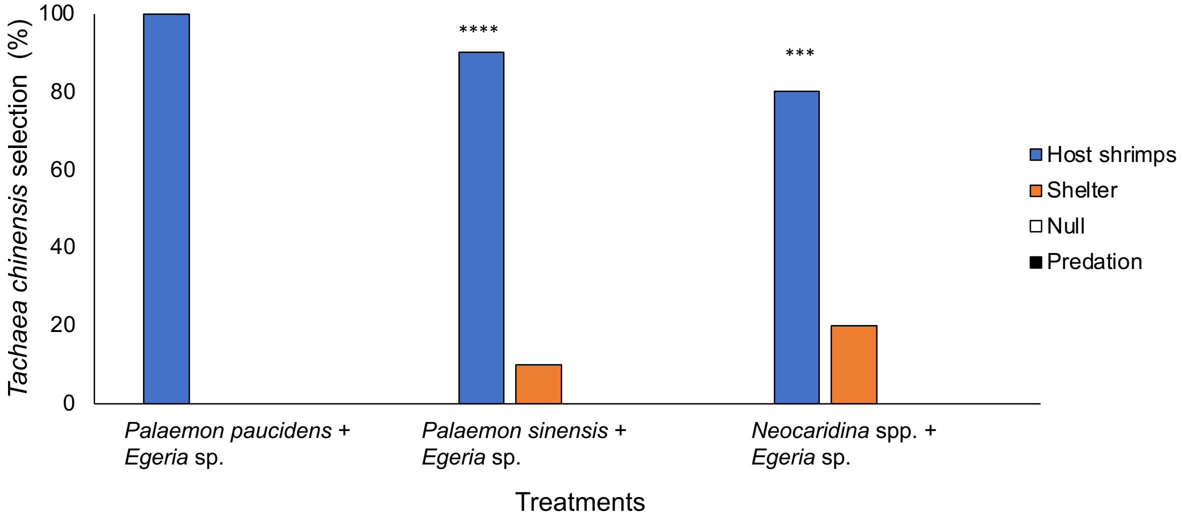


Figure 4.3: Selection percentage of *Tachaea chinensis* in the food vs shelter experiment. A sample size of 10 were used for each treatment; ***: $P < 0.001$ (Binomial of significance).

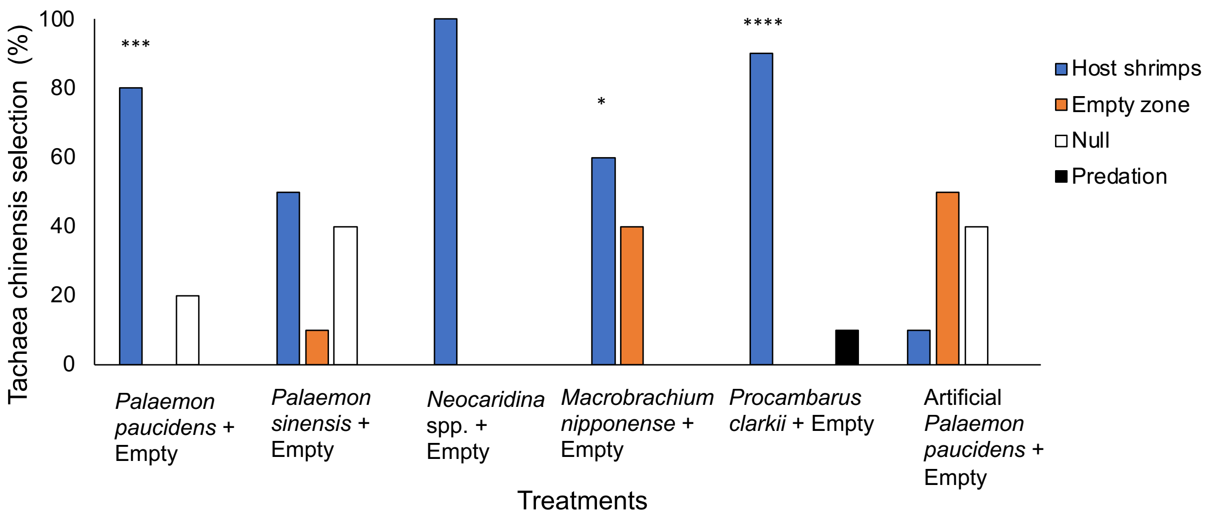


Figure 4.4: Selection percentage of *Tachaea chinensis* in the no-choice experiment. A sample size of 10 were used for each treatment; **: $P < 0.01$ (Binomial of significance).

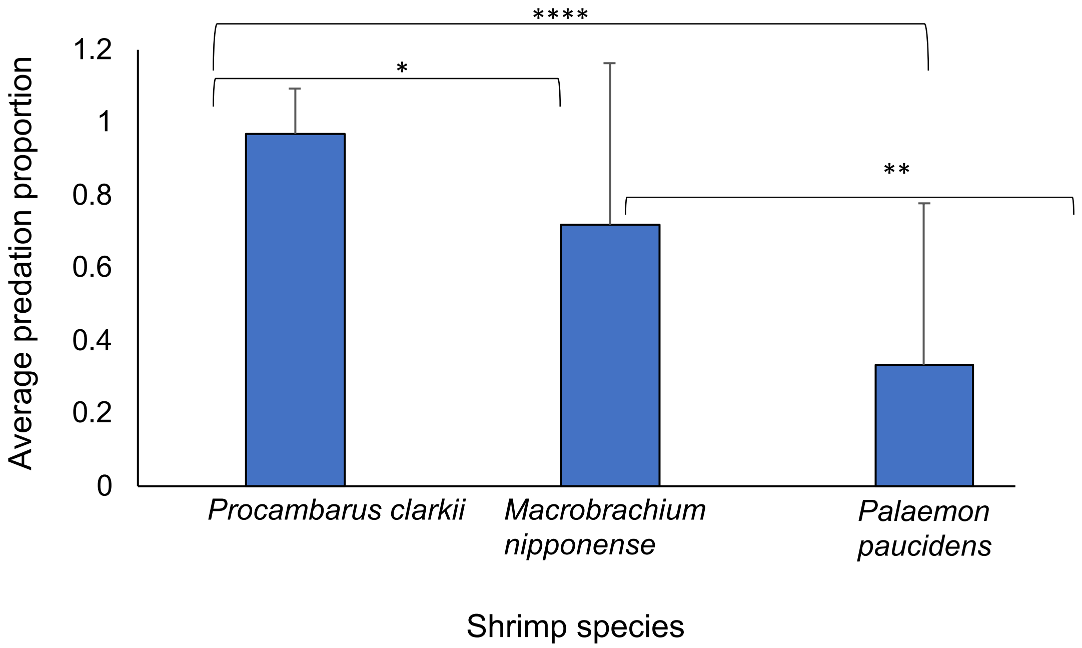


Figure 4.5: Average predation proportion of *Tachaea chinensis* in each shrimp species treatment. Fishers exact test, * $P < 0.05$, ** $P < 0.01$, **** $P < 0.0001$.

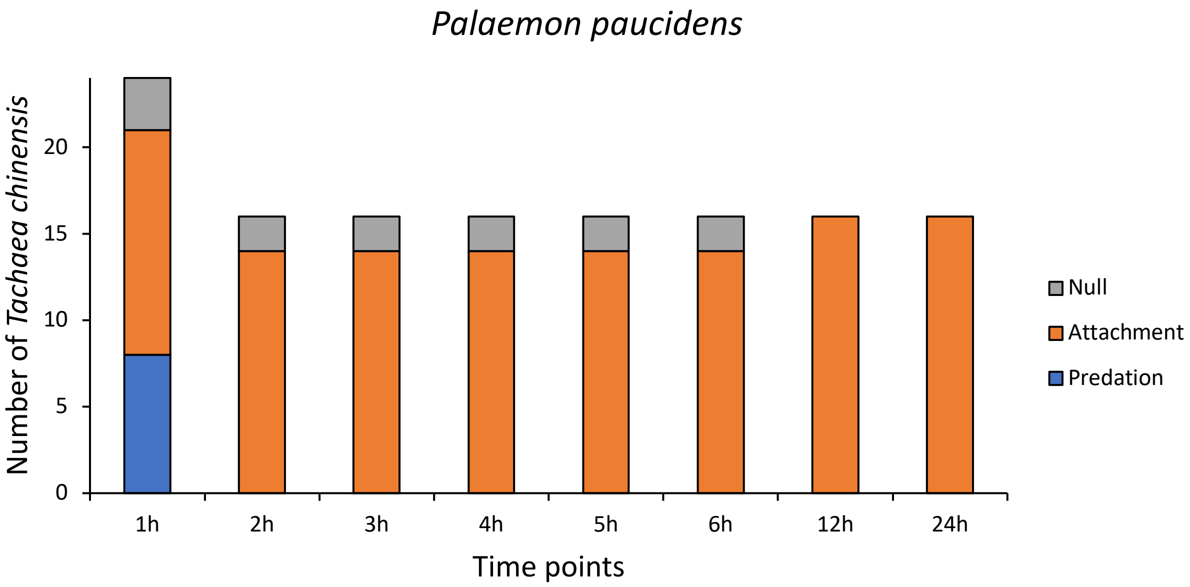


Figure 4.6: Number of *Tachaea chinensis* predated by *Palaemon paucidens*. Single *Palaemon paucidens* was provided in the tank (n=14). Two *Tachaea chinensis* were added in each tank.

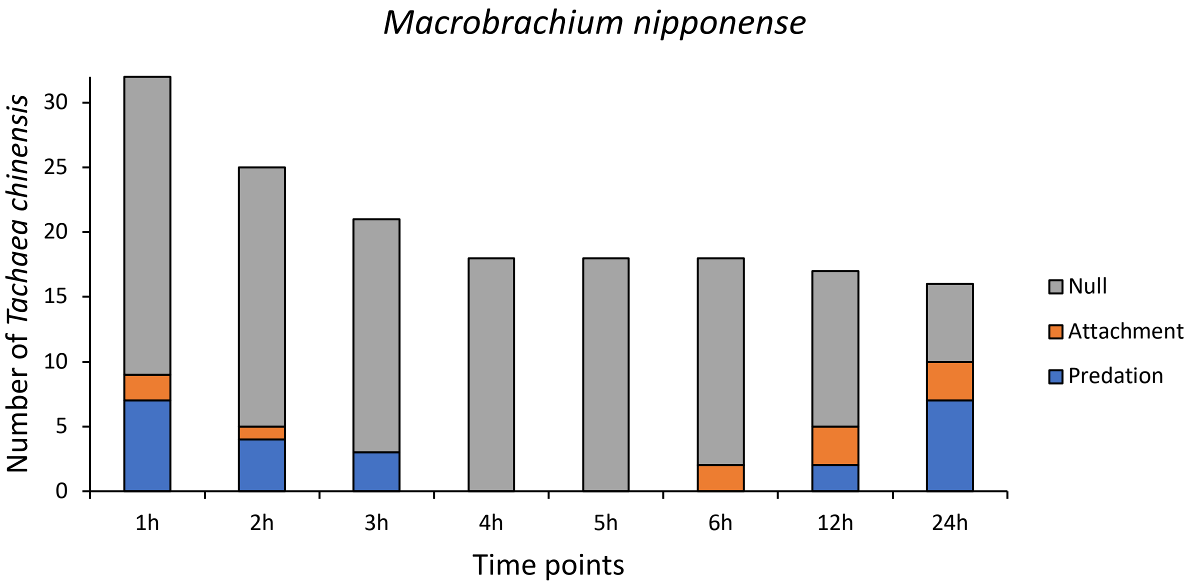


Figure 4.7: Number of *Tachaea chinensis* predated by *Macrobrachium nipponense*. Single *Macrobrachium nipponense* was provided in the tank (n=16). Two *Tachaea chinensis* were added in each tank.

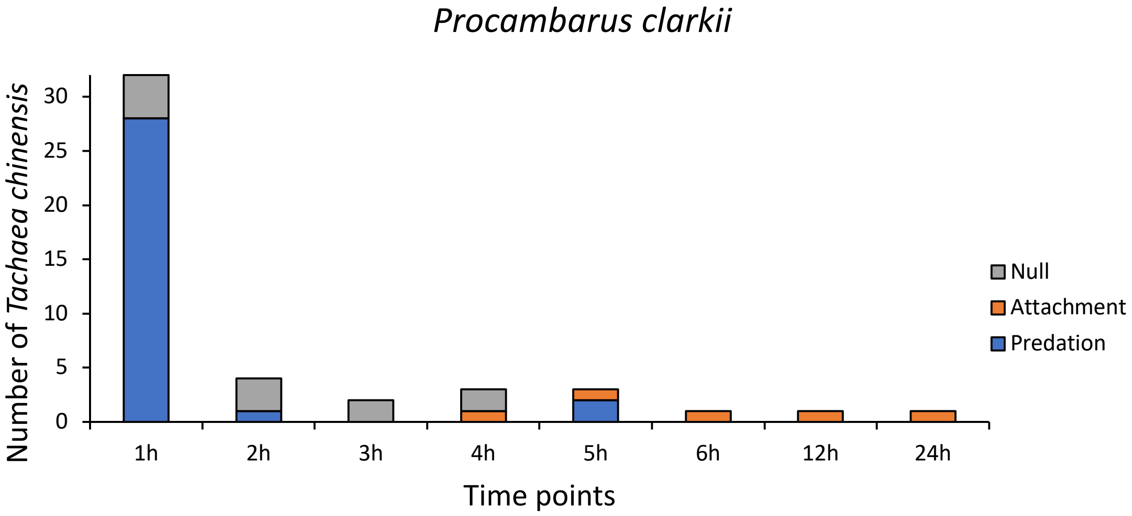


Figure 4.8: Number of *Tachaea chinensis* predated by Crayfish: *Procambarus clarkii*. Single *Procambarus clarkii* was provided in the tank (n=16). Two *Tachaea chinensis* were added in each tank.

4.4 Discussion

4.4.1 Un-common hosts' experiment

The isopod *T. chinensis* is known to infest a wide range of potential hots including the families; Atydae, Palaemonidae and Penaeidae (Xu et al. 2019; Nagasawa et al. 2018). The host-parasite interactions are usually restricted by many biotic and a biotic factor (Nagel and Grutter 2007). Therefore, the observed associations between *T. chinensis* and host species may not necessarily correspond to low host specificity of the isopod. The current study is the first to investigate the host preference of this isopod using laboratory choice experiments.

T. chinensis demonstrated a significant host preference behavior towards the common host *Palaemon paucidens* in treatments involving the uncommon hosts (*Macrobrachium nipponense, Procambarus clarkia,* artificial *Palaemon paucidens* and Fish). Similar to field studies *Palaemon paucidens* was found to be the preferred host for the isopod, although when less abundant, it might utilize *Neocaridina* spp. as an additional host (Nagasawa et al. 2018). The reason behind the preference towards these two host shrimps is yet to be explored, although an explanation could be drawn from the symbionts-host genetical imprinting. This hypothesis implies that isopods might be genetically coded to select the favorable host shrimp at younger stages and/or to favorer the source host upon which the parasite is retrieved. While later stage preferences could be governed entirely by different factors including; quality of food, low predation probability and/or availability of space (Nagel and Grutter 2007). However, this theory needs further elucidation as manca stage isopods were found randomly selecting easily accessible host shrimps at first encounter in this study and other studies (Xu et al. 2021).

To a lesser extent the isopod chose crayfish and fish in this study, whereby the latter being less common part of this association in nature. This isopod is occasionally found on the common carp, *Chanodichthys erythropterus* in China (Shen 1936). Similar fish species were not found around the collection sites of *T. chinensis* in this study. Nonetheless, occurrence of these isopods on fish is generally regarded as accidental (Nagasawa et al. 2018).

Surprisingly however, although attachments incidences were witnessed in all shrimp's species treatments, the young Crayfish *Procambarus clarkii* as well as *Palaemon paucidens* and *Neocaridina* spp. appear to be a preferred option for the isopod compare to *Palaemon sinensis* and *Macrobrachium nipponense* shrimps in the no-choice experiment. In Japan, *T. chinensis* has been found infecting 9 different species of shrimps at various localities (Nagasawa et al. 2018). The same study also found that, the preferred host for the isopod varies between the main islands and others islands, as shrimps' fauna in the latter varies greatly. Indicating that these isopods are generalist parasite and could adapt and feed on different host shrimps depending on its geographical location. This study is first to report the potential infestation of *T. chinensis* on the young crayfish *Procambarus clarkii,* providing an additional source host to the total already known host shrimps of this isopod in Japan (e.g., 9 host shrimps species). Therefore, these treatments demonstrated that *T. chinensis* isopod could show a great level of flexibility and adaptation in regards to the host species and habitat selection as supported by previous research on the isopod (Ota 2019; Xu et al. 2019).

4.4.2 Food and shelter Experiment

Isopods of the geneus *Tachaea* spp. is commonly found attaching on the carapace of the host shrimps and as symbionts to sponges (Xu et al. 2019). Once a secure attachment is made, the isopod start feeding on the host blood and mucus using their sophisticated mouth parts (Li et al. 2021). The young are suggested to live on the host the entire period of infestation, while later stages will thereafter detach heading towards rocks and dead plants for reproduction (Ota 2019). Hence, temporary shelters could resemble a safe zone from predators, therefore ensure successful recruitments. In this study, freshwater submerged plants were used as temporary shelter in pairwise experiments investigating the choice between food and shelter. The results revealed a significant selection towards the host shrimps instead of the provided shelters. Possibly indicating the importance of food for the isopods as they were starved for 24 hours prior experimenting. Supporting previous reports suggesting the feeding of this isopod on the mucus and hemolymph of the associated host using specialized mouthparts (Li et al. 2021). Additionally, the tested isopods may not be reproductively mature hence there is no obligation to search for a shelter at this point of their life cycle. However, such interactions are rarely reflecting the real conditions in the wild where in-hand sheltering options and elevated chance of predation are always available (Xu et al. 2021). Therefore, further research including the use of in situ pairwise experiments are needed to fully understand the selection mechanisms of the isopod during different stages of their life cycle. Moreover, additional research is needed to identify the chemical and visual attractants potentially produced by host shrimps, that grants isopods observed selections. As other isopods were documented to prefer/avoid specific chemical and/or visual cues (Sehr et al. 2016).

4.4.3 Predation

Little is known about the fate of the host-parasite interaction in regards to host predation. A single previous observation was previously noted by Xu et al. (2021) in China, whereby manca stage isopod was seen consumed by the host shrimp in laboratory condition. The current study is the first to explore the potential predation of this relationship between the isopod and the associated host shrimps in laboratory conditions. All of the tested host shrimps were found preying on *T. chinensis* isopods at varying degrees. Other reports demonstrating the predation of the host on different species of isopods were also documented (e.g., gnathiid isopods) (Penfold et al. 2008). Compared to *Macrobrachium nipponense* and *Palaemon paucidens* shrimps, *Procambarus clarkii* crayfish consumed more isopods in the current study. The crayfish shrimp even ate the offered isopods in a lesser time frame (1h). This species in native to north America. It has been introduced to Japan in in 1927, upon which it demonstrates a great adaptability and expanded its locality in nature (Kawai and Kobayashi 2005). Its destructive abilities include the consumption of freshwater plants and macroinvertebrates which ultimately leads to the reduction of biodiversity in the specific habitat (Souty-Grosset et al. 2016). In addition, they were seen feeding on specific size class of these organisms (Machida and Akiyama 2013). Therefore, making it more experience in consuming other species compared to the other tested shrimps. Although the minimum size difference between host and parasite that would trigger predation was not the aim of the study. The results tend to reflect that, the greater the difference between the host and parasite, the greater is the predation probability. Moreover, the strength and presence cheliped could not be ignored as it might govern the initiation or inhibition of predation behavior between the two organisms. However, such suggestion needs further investigations in the future in order to fully understand the basis behind predation and/or the consequence of such act on isopods population dynamics.

4.4.4 Conclusion

In conclusion, the current findings revealed that, in the presence of common and uncommon host shrimps, the isopod showed a significant selection behavior towards the common host. In addition, *T. chinensis* isopods was clearly attracted towards host shrimps compared to shelter place indicating the important of food at this current life stage. The low host specificity however, complies with previous studies on other parasitic isopods as well as on *T. chinensis (*Gentil-Vasconcelos and Tavares-Dias 2015; Ho and Tonguthai 1992; Xu et al. 2021; Ota 2019; Nagasawa et al. 2018). In regards to host predation on parasite, this study clearly showed the potential consumption of isopods by the tested hosts. Despite consumption was observed in all of the three tested shrimps, the invasive crayfish *Procambarus clarkii* consumed greater percentage in shorter time. Suggesting an increased predation on the isopods upon existing in crayfish favorite habitats. In this study we demonstrate the host specificity and predation of isopods in laboratory conditions, therefore, further investigation should focus on the fate of manca stage isopods on these aspects of the host parasite interaction in order to fully understand the season prevalence and population dynamics.

Chapter 5

Effects of the ectoparasite *Tachaea chinensis* on the growth condition and gonads development of the freshwater shrimp *Palaemon paucidens*

5.1 Introduction

The order Isopoda (Crustacea) is a home to both free-living and parasitic species. Many of which are found in the freshwater, marine, and even terrestrial environments. They can be found in association with fish, crustaceans and, in rare cases, echinoderms (Poulin 1995). However, the degree of the symbiotic living mode varies greatly among parasitic isopods. Some are permanent (e.g., Cymothoids and Gnathiids) whereas others are temporary or accidental (e.g., Aegids, Cirolanids and Corallanids) (Bunkley-williams and Williams 1998).

Parasitism by isopods from the genus *Tachaea* has been shown in many species including; freshwater shrimps, sponges and fish (Delaney 1989; Mariappan et al. 2003; Sahoo et al. 2022; Ota 2019). These parasites cause various detrimental effect to their associated host. For example; *Tachaea chinensis* has been shown to alter the energy levels and the visual system in *Palaemonetes sinensis* (Yu et al. 2021). While it was also responsible for the inhibition of the antioxidant and immune response as well as placing a burden on the glucose metabolism process of *Palaemon sinensis* (Li et al. 2019; Li et al. 2020). Moreover, secondary infections (e.g., *Vibrio* sp.) attracted by *Tachaea chinensis* lesions has been also documented in the literature (Ueki et al. 1988). However, whether or not these negative effects are momentary and/or could subsides during the dislodgment of the parasite remained to be investigated.

Tachaea chinensis isopods are commonly found infesting shrimps and prawns in Japan, whereby *Palaemon paucidens* being the preferred host for the parasite in temperate regions (Nagasawa et al. 2018). In Japan, *Palaemon paucidens* are distributed in rivers, ponds and lacks (Nishino 1980). It is ecologically important and gained a commercial value via their use as fishing baits by the fishermen (Imai and Oonuki 2014). Despite the exploitation for economical proposes, *Tachaea chinensis* parasite could provide another stressor to these shrimps affecting their growth and recruitment in nature. Until now alteration in the reproductive development and body condition of *Palaemon paucidens* in response to parasitism remained unclear. Therefore, in this study we investigated the effect of *Tachaea chinensis* isopods on the gonadal development and body condition of lake prawn *Palaemon paucidens* in Shimane and Okayama prefectures.

5.2 Materials and methods

The Shrimp *Palaemon paucidens* and isopod *Tachaea chinensis* were collected using hand nets (2.5- mm, 35 x 35 cm opening), from rice field waterways and ponds at Shimane prefecture and Okayama prefecture during April 2022. The net was lowered around the submerged vegetation and water-ways walls between 10 – 60 cm depth range. Specimens were placed in coolers fitted with portable aerators and transported to the laboratory of Aquatic Ecology at Hiroshima university for further processing.

A total of 59 and 84 samples were obtained from Shimane prefecture and Okayama prefecture respectively during April 2022. The sampled shrimps were then sorted, according to *Tachaea chinensis* infestation, to; 1) infested and, 2) non-infested groups. All specimens were kept in separate buckets fitted with aerations for at least 24 hours, without food, before subsequent analysis. The water in the buckets was replaced every two days to avoid water quality deterioration. Shrimps were then sampled and anesthetized in ice bucket for approximately 20 minutes before dissecting and examination.

Specimens were sorted according to sex by examining the presence or absence of appendix masculine on the exopod of the second swimmeret (Anger and Moreira 1998). Carapace length (CL) was measured from the base of the eye stalk to the posterior margin using a digital caliper (0.01 mm). For each specimen, the wet-weight measurements of the shrimp's body (BW), the gonads (GW) and the hepatopancreas (HW) were measured to the nearest 0.0001 g using an analytical balance. From which, the gonadosomatic index (GSI) and the hepatosomatic index (HSI) were calculated using formula (1) and (2):

$$\text{GSI} = (\text{Wet gonad weight} / \text{Wet body weight}) \times 100 \qquad (1)$$

$$\text{HSI} = (\text{Wet hepatopancreas weight} / \text{Wet body weight}) \times 100 \qquad (2)$$

In addition, a total of 217 shrimps' specimens were examined. These samples were obtained during April through June 2018 from Shimane prefecture and were preserved in 70 % ethanol. Shrimps were analyzed for the difference in the overall health and body condition. The relative condition factor (K_n) for each individual was calculated using the equation $K_n = W/aL^b$ (for an allometric growth pattern, $b \neq 3$). Where W; is the wet body weight, L; is the carapace length, a; is the y- intercept and b; is the slope calculated from the length-weight relationship analysis.

5.2.1 Statistical analysis

Data were tested for its normality using the Shapiro-Wilk test. A linear regression analysis was performed to evaluate the energy cost during the reproductive season of the shrimp between the three group. The Kruskal-Wallis's test of significance was used for multiple comparison in the mean GSI and/or HSI between non-infected and infected shrimps. All statistical tests were performed using R Statistical software (Version 4.0.3, R foundation for statistical computing, Vienna, Austria) and Microsoft Excel software (Version 16.57, 2019).

5.3 Results

A total of 360 *Palaemon paucidens* shrimps (143 and 217 live and preserved samples, respectively) were examined in this study. Out of which, 170 samples were found infested by *Tachaea chinensis.* The carapace length of all sampled shrimps ranged between 4.84 mm to 14.04 mm. Infestation prevalence was found 48.2 % in females and 51.8 % in male shrimps (Table: 5.1 –5.3).

There was no significant different in the mean GSI observed between infected and non-infected samples in Shimane prefecture and Okayama prefecture ($P > 0.05$) (Figure 5.1 and Figure 5.3). Similar results were obtained in regard to the relative condition factor (K_n), in which no significant difference was recorded between infected and non-infected groups of shrimps (Figure 5.2, 5.4 and 5.5).

The body weight to carapace length relationship revealed a negative allometry ($b< 3$) in all sample groups, except for infected females and infected males collected from Okayama prefecture and Shimane prefecture respectively. The linearized equation for each group in each sampling site can be seen in tables 5.1 –5.3.

Table 5.1: Body weight – carapace length relationship, Growth pattern and mean relative condition factor for preserved infected and non-infected *Palaemon paucidens* samples collected from Shimane prefecture during April 2018.

		n	Linearized Equation ln y = ln a + b ln x	R^2	Slop (b)	Growth Pattern	Mean Kn + SD
Infected	All samples	52	logWeight = -2.821 + 2.555log CL	0.89	2.55	(-)Allometry	1.016 (0.161)
	Males	23	logWeight = -2.519 + 2.186log CL	0.8	2.18	(-)Allometry	1.023 (0.183)
	Females	29	logWeight = -3.014 + 2.773log CL	0.91	2.77	(-)Allometry	0.974 (0.118)
Non-Infected	All samples	35	logWeight = -2.830 + 2.589log CL	0.9	2.58	(-)Allometry	0.995 (0.151)
	Males	22	logWeight = -2.698 + 2.441log CL	0.85	2.44	(-)Allometry	1.011 (0.149)
	Females	13	logWeight = -2.966 + 2.733log CL	0.931	2.73	(-)Allometry	0.992 (0.153)

Table 5.2: Body weight – carapace length relationship, Growth pattern and mean relative condition factor for preserved infected and non-infected *Palaemon paucidens* samples collected from Shimane prefecture during May 2018.

		n	Linearized Equation ln y = ln a + b ln x	R^2	Slop (b)	Growth Pattern	Mean Kn +SD
Infected	All samples	50	logWeight = -2.848 + 2.551 log CL	0.916	2.55	(-)Allometry	1.023 (0.152)
	Males	24	logWeight = -2.809 + 2.533log CL	0.892	2.53	(-)Allometry	1.039 (0.127)
	Females	26	logWeight = -3.052 + 2.746log CL	0.933	2.74	(-)Allometry	0.995 (0.153)
Non-Infected	All samples	18	logWeight = -2.905 + 2.672log CL	0.946	2.67	(-)Allometry	1.045 (0.134)
	Males	9	logWeight = -2.793 + 2.568log CL	0.954	2.56	(-)Allometry	1.009 (0.108)
	Females	9	logWeight = -3.615 + 3.393log CL	0.974	3.39	(+)Allometry	1.214 (0.0991)

Table 5.3: Body weight – carapace length relationship, Growth pattern and mean relative condition factor for preserved infected and non-infected *Palaemon paucidens* samples collected from Shimane prefecture during June 2018.

		n	Linearized Equation ln y = ln a + b ln x	R^2	Slop (b)	Growth Pattern	Mean Kn + SD
Infected	All samples	25	logWeight = -2.877 + 2.632 log CL	0.967	2.632	(-)Allometry	1.025 (0.098)
	Males	10	logWeight = -2.695 + 2.420log CL	0.928	2.42	(-)Allometry	1.00 (0.068)
	Females	15	logWeight = -2.826 + 2.587log CL	0.956	2.587	(-)Allometry	0.998 (0.108)
Non-Infected	All samples	37	logWeight = -2.9497 + 2.742log CL	0.948	2.742	(-)Allometry	1.03 (0.161)
	Males	16	logWeight = -2.684 + 2.464log CL	0.742	2.464	(-)Allometry	0.996 (0.150)
	Females	21	logWeight = -2.915 + 2.685log CL	0.958	2.685	(-)Allometry	1.023 (0.155)

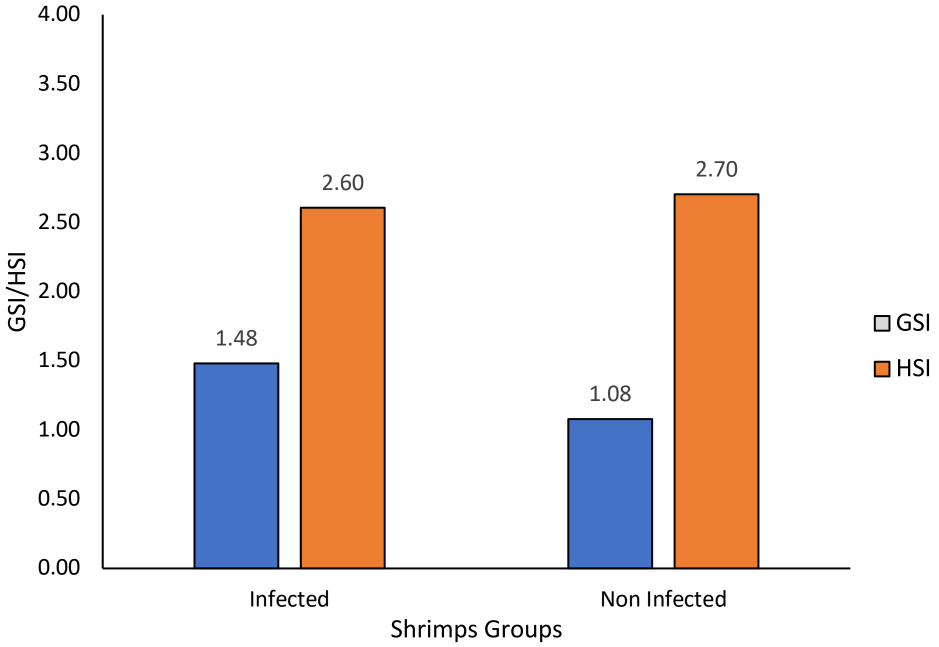


Figure 5.1: Average Gonadosomatic indexes (GSI) and Hepatosomatic indexes (HSI) of infected and non-infected *Palaemon paucidens* collected from Shimane prefecture (n = 59).

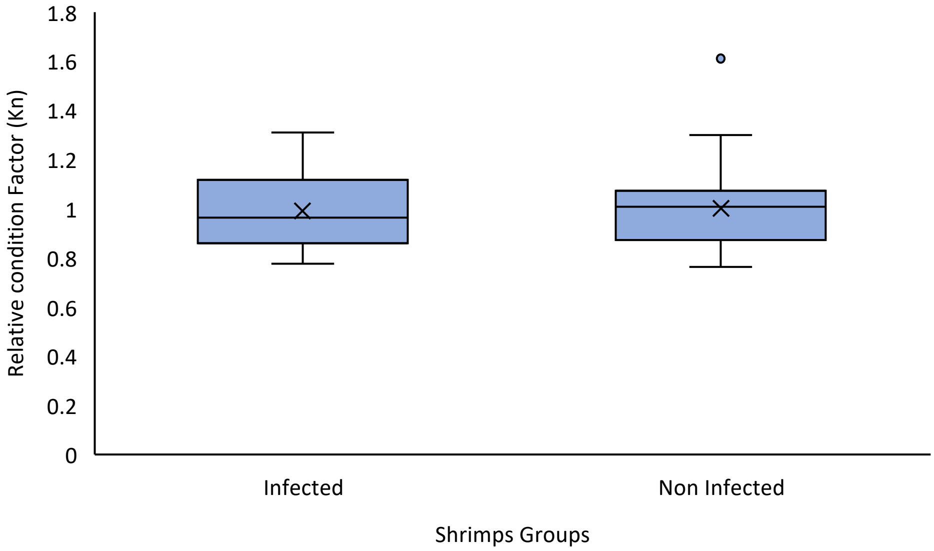


Figure 5.2: Boxplot of the relative condition factor (K_n) for infected and non-infected *Palaemon paucidens* collected from Shimane prefecture. Boxplots are combined with a mean, minimum, maximum, first quartile, median and third quartile values.

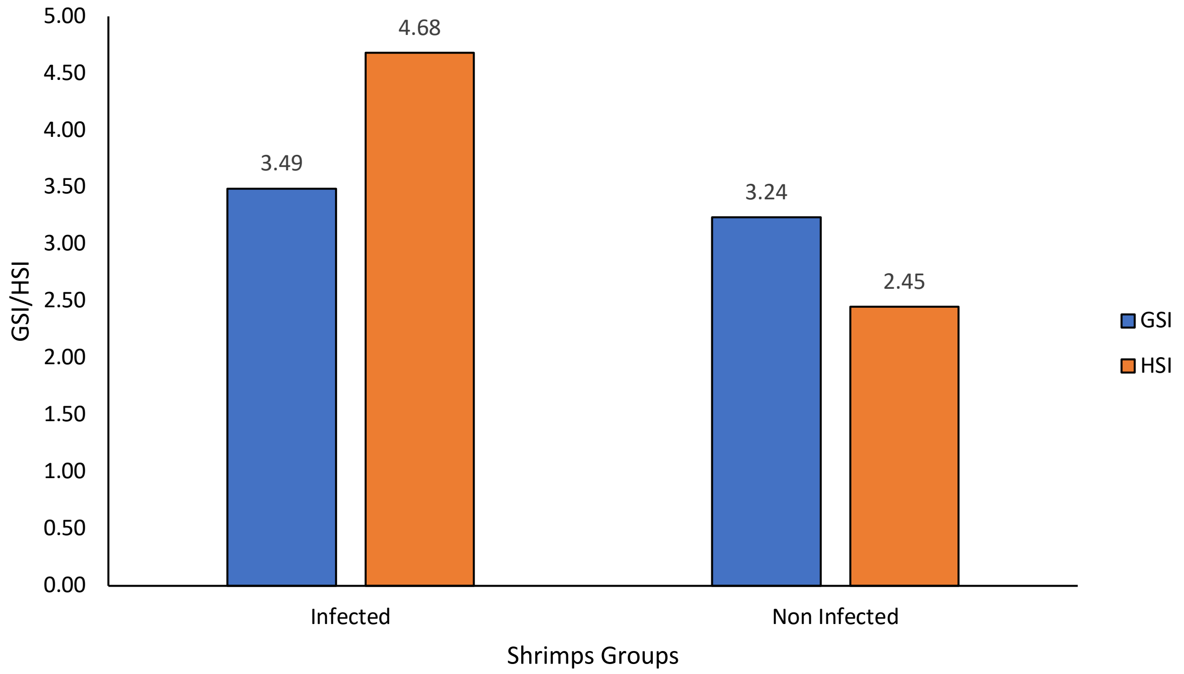


Figure 5.3: Average Gonadosomatic indexes (GSI) and Hepatosomatic indexes (HSI) of infected and non-infected *Palaemon paucidens* collected from Okayama prefecture (n = 84).

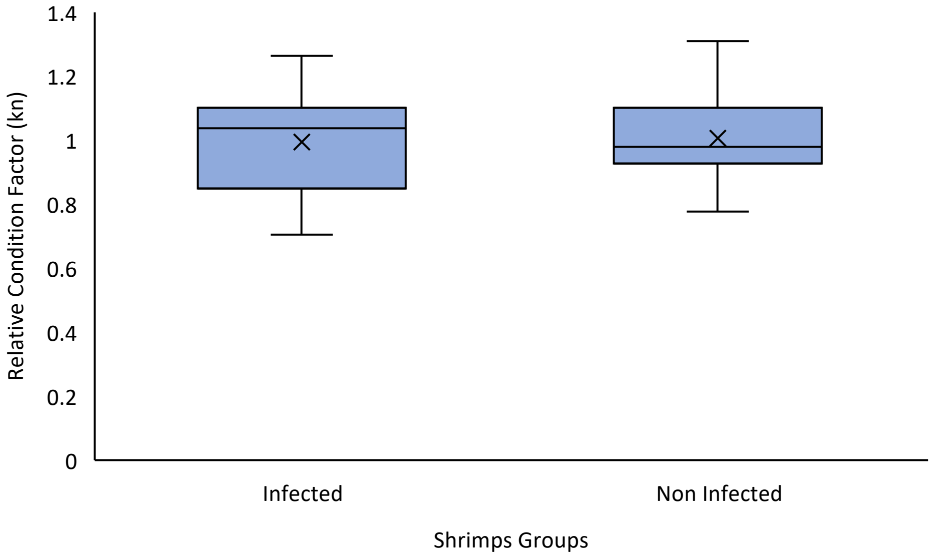


Figure 5.4: Boxplot of the relative condition factor (K_n) for infected and non-infected *Palaemon paucidens* collected from Okayama prefecture. Boxplots are combined with a mean, minimum, maximum, first quartile, median and third quartile values.

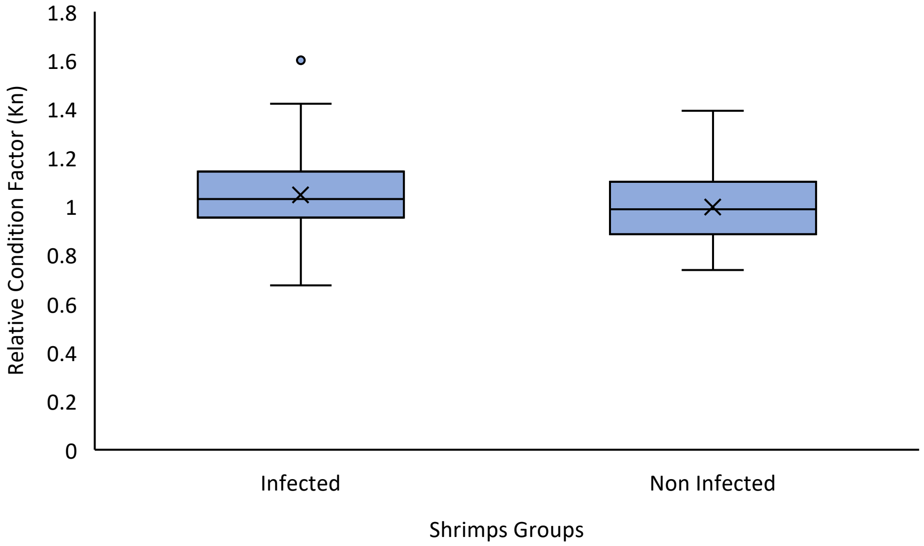


Figure 5.5: Boxplot of the relative condition factor (K_n) for preserved infected and non-infected *Palaemon paucidens* samples collected from Shimane prefecture during April through June 2018. Boxplots are combined with a mean, minimum, maximum, first quartile, median and third quartile values.

5.4 Discussion

Parasitic isopods cause various effects on their associated hosts. These effects could range from direct (e.g., stress, tissue and organ damage, inhibition of embryonic development, blood loss and osmoregulatory problems) to indirectly affecting the hosts; as vector to various pathogens (Rameshkumar and Ravichandran 2014; Ueki et al. 1988; Calado et al. 2005). However, records of the isopods bearing minimum to non-significant effects had also been observed. For example, the Bopyrid isopod *Parabopyrella* sp. has been shown to affect the quantity but not the quality nor being responsible for reproductive death of its host shrimp (Calado et al. 2006). Similarly, the parasitic isopod *Ichthyoxenus japonensis* excreted no difference in the body to weight ratio and the condition factor between the infected and non-infected host (Hua et al. 2017). The results from the current study revealed no significant difference in the GSI and HSI nor the body condition between infected and non-infected host shrimps *Palaemon paucidens.* Suggesting, minimum effects excreted to the gonadal development and growth of these shrimps. Parasitized females (Egg-bearing) were frequently recorded in this study. However, despite parasitism, some were even found at advance stages of embryonic development (eyed-eggs). Possibly, the effects are more prominent in aspects including energy utilization, enzyme inhibition and/or the quantity and quality of developed embryos, an area beyond the scope of the current study. It is also possible that the effect may become more apparent depending on the length of the infection period. Li et al. (2020) suggested that *T. chinensis* could negatively affects the antioxidant and immune response at prolonged stages of infection (> 15 days). However, the initial upregulating of certain enzymes could be a temporary response to infestation, which subsequently shown to decrease in that study.

Moreover, *T. chinensis* is a temporary parasite spending approximately 10 months attached on the host shrimp before it presumably detaches during the mating season (Ota 2019; Khalfan et al. 2022). It is therefore suggested that the negative effect of infestation could possibly be reversed in the resume of feeding of the host and therefore the resume of growth and energy, during the absence of parasites (Lozano 1991). Thus, further studies are needed to examine the effect of prolonged parasitism periods in the future. The mating behavior *Palaemon paucidens* is characterized by males clinging and/or wrapping to the anterior section of the females (Ogawa and Kakuda 1988). Therefore, *T. chinensis,* if present, hypothetically could interfere with such mating behavior. However, the physical inhabitation of the mating behavior of *Palaemon paucidens* in the presence of isopod attachment remained to be explored.

In conclusion, *T. chinensis* has been observed to infect freshwater and brackish water shrimps and prawns. Increased infestation by this species was shown to cause kills in cultured shrimps in China (Xu et al. 2019). Until now, information on the mechanism behind the effect of these isopod on their host shrimp is still lacking. We investigated the passible effect of *T. chinensis* on the body condition and gonadal development of *Palaemon paucidens* shrimps. The results indicated that, *T. chinensis* excreted no significant effect on the growth nor the gonadal development of infected shrimps. Therefore, we suggest that longer duration of infection could reveal a significant effect on the energy levels and the reproductive output of the infected shrimps.

Chapter 6

General Discussion

Tachaea chinensis isopods are ectoparasites of freshwater shrimps and prawns. There are often found attached to either side of the carapace of its associated hosts. Presumably causing metabolic stress and secondary pathogenic infections on the infected shrimps (Ueki et al. 1988; Li et al. 2019a; 2019b). Previous research has been limited to the biogeography, taxonomy and ecology of *Tachaea chinensis*, where little attention has been devoted to the behavioral and reproductive effects caused by these isopods on host shrimps. The understanding of population dynamics of these ectoparasites and the establishment of an effective infection mitigation measure, requires an in-depth mechanical understanding of their behavior and selection pattern (Cook and Munguia 2013; Olabarria et al. 2002). Therefore, this study investigated the size selection behavior of *Tachaea chinensis* isopods using field and laboratory experiments, shed light on the isopod's host species specialization and examined the possible effects of *Tachaea chinensis* on the host's growth and gonadal development.

6.1 Size selection of *Tachaea chinensis* in nature

Results from chapter 2 showed that the body length of *T. chinensis* and the carapace length of the host were consistent 0.8 – 0.9. Indicating that the isopod continuously found on host shrimps whom are slightly larger than its own size as it grows. This is supported by Ota (2019) who suggested that *Neocaridina* spp. did not provide enough space for a large isopod to attach. The results also showed that the body length of *T. chinensis* was an important determinant of its host species. Sampled isopods that are less than 6 mm parasitized both *P. paucidens* (37.5 –96.2%) and *Neocaridina* spp. (3.8 – 62.5%), however, *T. chinensis* isopods larger than 6 mm were found almost exclusively infesting *P. paucidens* shrimps. Suggesting field evidence of the presence of size selection in the isopoda *T. chinensis*. Whether or not the parasite grows alongside with host growth, dislodgments of ectoparasites often occurs during the death of the host (Xu et al. 2021), therefore locating and selecting another appropriate host after dislodgment incidences is timely vital. The mean carapace length of both *P. paucidens* and *Neocaridina* spp. was found to be less than 6 mm in August and September in 2018 and 2019. However, *P. paucidens* carapace length rapidly exceeded 6 mm from October to June. Indicating the presence of a comparable growth rate between the two shrimp's taxa, which could ultimately trigger a size selection behavior in this isopod in nature. *T. chinensis* might ectoparasitize both shrimps at younger stages but likely to show size preference as it grows. Further research is needed using wild investigations including behavioral observations of Manca stage *T. chinensis* isopod.

6.2 Size selection of *Tachaea chinensis* in laboratory condition

Under laboratory conditions, *T. chinensis* demonstrated a level of recognition towards the large host sizes. A total of 70 isopods were allowed to select between host shrimps of different size and different species in pairwise selection experiments. Higher percentage (87.1 %) of the isopods were found attached to the carapace of the provided host options. In treatments involving different sizes of the same host species (*P. paucidens, Neocaridina* spp. and *P. sinensis)*, *T. chinensis* tended to select the larger host in two out of three treatments. These findings complimented the results obtained from chapter 2 (e.g., size selection in nature). Ectoparasite isopods tend to only grow to a level that provide protection from accidental dislodgment during molting (e.g., crustaceans) or swim drag (e.g., fish) caused by the host (Poulin 1995). Therefore, smaller host shrimps could provide less favorable space for large isopods to safely attach (Ota 2019).

It is suggested that *T. chinensis* distribution might be influenced by the abundance of host shrimps at a given geographical site (Nagasawa et al. 2018). However, the number of available host shrimps for the isopods were held equal in all trials. Therefore, species abundance was hardly the only determinant factor for the selection made by the isopods. Moreover, stimulants such as; visual, chemical and tactile were all presents, demonstrating that this isopod might use multiple sensory cues to locate suitable host in the tank. Further studies amid at the investigation of sensory cue's efficacy in these isopods are needed. *T. chinensis* demonstrated greater preference towards larger host option (90 %) when provided with mixed host options (*Palaemon paucidens vs Neocaridina* spp.). The reason behind the null results of *T. chinensis* in treatments involving *P. sinensis* is unknown, however, we suspect that the exotic *P. sinensis* might be more active and thus could easily escape infestation compared to *P. paucidens and Neocaridina* spp. Incidence of isopods host switching was observed between 3 h and 18 h time points in this study. It is also possible that, this behavior demonstrated by few isopods is temporary and beyond the experiment time scope (18 h). However, such hypotheses remained to be tested in the future.

Size selection could be different at different life stage of the parasitic isopod. Therefore, smaller individuals might select a larger space that would not restrict their growth in the future (Welicky et al. 2019). However, results from Manca stage treatment demonstrated otherwise. Manca staged isopods were seen attached randomly to both large sized and small size *Neocaridina* spp. host shrimps. Similar findings were observed by Xu et al. (2021), where Manca stage isopods showed low host specificity in laboratory condition. The reason behind this is yet to be explored, however, Coile and Sikkel (2013) found the host surface area was a significant predictor of ectoparasite gnathiid infestation patterns across different hosts. Therefore, we suspect that since both host options resemble enough available space, Manca stage isopod has little need to show a significant size selection behavior.

The current findings concludes that when large differences, in the surface area provided between host pairs exist, *T. chinensis* tend to demonstrate size preference behavior regardless of the host species, which ensures suitable space for attachment and growth. This result complies with Ota (2019) who indicated that the differences in prevalence of *T. chinensis* may be linked to the ontogeny speed and size at maturity of common *T. chinensis* in the natural habitat. Therefore, we suggest that *T. chinensis* likely to show size specificity according to the developmental stage.

6.3 Host species selection of *Tachaea chinensis* and host-parasite predation

T. chinensis ectoparasites has been found infesting a broad range of host shrimps, including 3 families of shrimps; Atydae, Palaemonidae and Penaeidae (Xu et al. 2019; Nagasawa et al. 2018). The wide range of potential hosts could be attributed, in part, to the abundance of host species and low parasite competition, which allows for infection in diverse group of freshwater shrimps (Nagasawa et al. 2018; Xu et al. 2019). While in the other part, it involves the great adaptability of this isopods to different habitats and host shrimps (Xu et al. 2019).

In this study *Tachaea chinensis* showed a great preference towards the common host shrimp *Palaemon paucidens* when subjected with uncommon host shrimps. Indicating, a significant degree of preference towards the commonly infested host shrimps. The reason behind such preference could be related to host genetical imprinting as the isopods used in this study were solely retrieved from *Palaemon paucidens* host shrimps. Host imprinting is a widely known phenomena in other decapods symbionts (Baeza and Stotz 2003; De Bruyn et al. 2011; Ory et al. 2003; Antokhina and Britayev 2020). However, further research is needed to elucidate this hypothesis in these parasitic isopods.

The majority of the tested isopods were found preferring host shrimps instead of shelter plants in this study. Suggesting host shrimps being the main source of food for theses parasites in nature. Ota (2019) also suggested that the isopod could infect host shrimp upon hatching while only detach for reproductive proposes. Similar findings were also observed under laboratory condition, as isopods become progressively weekend and subsequently died after the sixth day without host (Xu et al. 2021). However, the interactions between host shrimps and the isopods might be different in nature, where complex sheltering system and looming potential predators are available for both organisms (Xu et al. 2021). The results from this experiment also indicated that the host specificity of this isopod is low. These results comply with previous surveys on the distribution of the parasite in Japan (Nagasawa et al. 2018; Ota et al., 2019). Similar findings on host preference of manca stage *T. chinensis* was observed in laboratory conditions in China. *T. chinensis* were found attaching on *P. sinensis* and *Exopalaemon modestus* and to a lesser extent *Macrobrachium nipponense* and *Neocaridina* spp. (Xu et al. 2021).

The laboratory trials investigating the potential host predation on isopod, indicated the ability of host shrimps to consume *T. chinensis* isopod at various degrees. Although all of the tested shrimp ate the isopod, the invasive crayfish were found to consume more isopods at a faster time frame (within 1hour). The reason behind such predation could be related to the size difference between the parasite and the host in question, as incidence of host predation were also documented in other isopod species. The authors concluded that while both host (small and large) consumed the isopod, greater number of isopods were consumed by the larger hosts (Penfold et al. 2008). The invasive crayfish has been shown to consume aquatic plants, fish eggs, macroinvertebrates and ultimately cause biodiversity reduction at a given location (Souty-Grosset et al. 2016). The maximum attainable size and adaptability of this invasive shrimp is much larger than the other two shrimps, which hypothetically could aid in the elevated destruction to the surrounding macrofauna. Although further research is need to fully elucidated its impacts on *T. chinensis* recruitment pattern and population dynamics.

6.4 Effects of *Tachaea chinensis* ectoparasite on growth and reproduction of *Palaemon paucidens*

In chapter 5, the effect of *Tachaea chinensis* isopods on the gonadal development and body condition of lake prawn *Palaemon paucidens* in Shimane and Okayama prefectures was investigated. Although *Tachaea chinensis* isopods were known to cause metabolic and physical damage to the host shrimps (Ueki et al. 1988; Li et al. 2019a; 2019b), the effects of this ectoparasites on growth and the gonadal development was found non-significant. The gonadosomatic index (GSI), Hepatosomatic index (HSI) and the relative condition factor (K_n), for both infected and non-infected shrimps were found comparably similar. Parasitized egg-bearing females were frequently recorded in this study. Despite parasitism, they were found at advance stages of embryonic development (eyed-eggs). Possibly, the effects might be more prominent in aspects including interference of mating behavior, energy utilization, enzyme inhibition and/or the quantity and quality of developed embryos, an area beyond the scope of the current study. These results contradict the conclusions of other studies on the effects of parasitic isopods on hosts reproduction and growth (Gokoglu et al. 2020; Petrić et al. 2010; Fogelman et al. 2009). Possibly the effect may become evident depending on the length of the infection period. The negative effects of infestation could also be reversed to some extant via the resume of feeding from the host and therefore resume growth and energy, during the absence of parasites (Lozano 1991). *T. chinensis* is a temporary parasite spending approximately 10 months attached on the host shrimp before it presumably detaches during the mating season (Ota 2019; Khalfan et al. 2022). We suggest that longer duration of infection might reveal a significant effect on the energy levels and the reproductive output and mating behavior of the infected shrimps. Further investigation is needed to investigate the possible alteration in the mating behavior of *Palaemon paucidens* shrimps.

6.5 Conclusions

Host specificity of *Tachaea chinensis* isopods is believed to have evolved as a result of the locality of its existence and the presented monopoly across different host shrimps due to the absence of other parasitic competitors. Previous studies were focused on the biogeographical and taxonomical aspect of this spices, while little research has been devoted to the understanding of the host preference in regards to the host size and host species. Field and laboratory experiments in this study indicated the presence of size selectively in these ectoparasites. Analysis of wild samples demonstrated that the isopod was constantly found in host shrimps that are slightly larger than its own body length at a ratio between 0.8 – 0.9 between isopods body length and hosts carapace length. This trend, in size selection, were further elucidated in laboratory pairwise experiments, whereby *Tachaea chinensis* selected the larger option in each treatment regardless of host species. Size selection play an important role in the survival of these isopod, as size of the carapace of host shrimps might restrict their future growth and hence recruitment (Ota 2019). Host species preference were found consistent with previously publish literature (Nagasawa et al. 2018; Xu et al. 2019). Indicating a broader range of potential host, hence greater prevalence in the natural habitat. However, this study also demonstrated potential consumption of these isopods by larger host shrimps. An important and unfortunately overlooked aspect in the understanding of the parasite's population dynamics. Finally, this study found minimum significant effect on the growth and the gonadal development of infected shrimps, possibly the effects might be more prominent in areas including mating behavior, energy utilization, enzyme inhibition and/or the quantity and/or the quality of developed embryos. We suggest further investigations on the effect of these isopods on the host in order to understand the pressure imposed on these freshwater shrimps and to help in the establishment of mitigation measures in the future.

Research Summary

Caridean shrimps represent an important constituent of many estuarine and freshwater habitats. While many species are native, others being anthropogenically introduced to new habitats accidentally or via trades. They are used as fishing baits and as pet in the ornamental industries. The shrimp *Palaemon sinensis,* a species native to china, was accidentally introduced to the ecosystem through their use as fishing baits by the fishermen. Despite the ecological risks imposed by shrimp's habitat relocation, little is known about the effects excreted on shrimp's host-parasite relationship and parasite distribution. The isopod *Tachaea chinensis* (Family: Corallanidae), is an example of an ectoparasite infesting both shrimps and prawns in Eastern Asian countries. They are often found attached to the lateral side of hosts carapace presumably preying on the host during immature stages. Previous research had focused on Caridean shrimps' distribution and/or *Tachaea chinensis* localities, however, little is known about the interaction between the two in terms of preference and possible parasite effects on host species. Therefore, this dissertation addresses the host-parasite relationship (e.g., size selection and host specificity) between the isopod *Tachaea chinensis* and its associated host shrimps by means of examining wild specimens and conducting laboratory pairwise choice experiments. This study also investigated the possible effects caused by the isopod on the common lake prawn *Palaemon paucidens* growth and reproductive development. Chapter 2 is focused on the establishment of a relationship between the body size of *Tachaea chinensis* and the carapace length of its associated host shrimps. In order to understand the size selection behavior of this isopod in nature, a total of 944 *Palaemon paucidens* and 279 *Neocaridina* spp. were collected from Shiga prefecture, Japan. The size specificity of *Tachaea chinensis* were found different according to body size. Individuals less than 6 mm parasitized both *P. paucidens* (37.5–96.2%) and *Neocaridina* spp. (3.8–62.5%), whereas *T. chinensis* larger than 6 mm were found infesting only *P. paucidens* shrimps. The ratios of the body length of *T. chinensis* to the carapace length

of the host shrimp were almost constant (0.8–0.9) with host species and parasite's growth. Indicating that *T. chinensis* tended to attach to host shrimps whom carapace length was slightly larger than its own body length. In chapter 3, the trend of size selection in nature was further elucidated in laboratory conditions, in order to provide a mechanical understanding of the size selection behavior of the isopod. A total of 70 isopods were allowed to select between host shrimps of different size and different species in pairwise selection experiments. Although few isopods did select the smaller option, the parasite showed greater response towards the larger host option. Therefore, when provided with pairs of different host's carapace length, *T. chinensis* may select larger hosts shrimps that are slightly larger than their own body length. *T. chinensis* (≥ 5 mm) were suggested to show size specificity according to the developmental stage. However, Manca staged isopods were seen attached randomly to both large sized and small size *Neocaridina* spp. host shrimps indicating low host specificity of Manca stage *T. chinensis* in laboratory condition. These findings suggests that when large differences, in carapace length, between host pairs exist, *T. chinensis* express more size selection behavior regardless of the host species.

Chapter 4 is designed to investigate the host species specificity of *T. chinensis* and examine the potential predation on the isopods by host shrimps. In total 13 treatments were conducted in order to clarify the host specificity of the isopod using pairwise choice experiments. These treatments were further subdivided into three groups namely; uncommon hosts, food and shelter and no choice treatments. *T. chinensis* significantly selected the common host shrimp *Palaemon paucidens* between the presented host options. Similar results were observed when a choice between food (e.g., host shrimps) and shelter were offer to the isopod. However, *T. chinensis* selected all of the five hosts options during no choice treatment. This indicates a low host specialization in the isopod *Tachaea chinensis*, which presumably reflect the wide distribution of this species in different freshwater habitats. The results from predation trials showed a significant predation behavior by host shrimps towards the isopod (Fishers exact test, $P < 0.05$). Although all of the tested shrimp were able to consume the isopod the invasive crayfish *Procambarus clarkii* ate greater percentage in significantly shorter time frame (within 1 hour) (Fishers exact test, $P < 0.0001$). Demonstrating an additional destructive behavior of this invasive crayfish on the biodiversity of freshwater habitats. In chapter 5, the growth and the reproductive development of *Palaemon paucidens* in response to parasitism was examined. A total of 360 *Palaemon paucidens* shrimps were examined in this study. Out of which, 170 samples were found infested by *Tachaea chinensis.* Analysis of the gonadosomatic index (GSI) and the hepatosomatic index (HSI) revealed no significant difference in the mean GSI and mean HSI observed between infected and non-infected samples in Shimane prefecture and Okayama prefecture ($P > 0.05$). Similar findings were observed in regards to the mean relative condition factor (K_n) between infected and non-infected samples. Despite parasitism, egg-bearing females were frequently recorded in this study. This could suggest a more prominent effects might arise in biological aspects of the isopod including; energy utilization, enzyme inhibition, pathogens infection and/or the quantity and quality of developed embryos. It is also possible that the effect may become more obvious in accordance to the length of the infection period. These results concluded minimal effects of *Tachaea chinensis* isopods on the growth and the reproductive development of its associated host shrimp *Palaemon paucidens.*

In conclusion, this study revealed that *Tachaea chinensis* are likely to demonstrate size selection behavior during the initial infestation on host shrimps, however, the fate of advanced stages might be governed by the other factors including; available space, risk of predation and other biotic factors. The host specificity of the isopod is suggested to be low, infecting a broad range of host shrimps. These results comply with previous studies on *Tachaea chinensis*, hypothetically this flexibility in regards to host shrimps' aids in the spread of these isopods in different habitats. In this study, the potential effects on hosts and predation of *Tachaea* *chinensis* by larger host shrimps were also documented. Providing a better understanding on the host-parasite interactions, which help in further research on the population dynamics of these isopods and hence the establishment of mitigation measures in the future.

References

Acquistapace P, Aquiloni L, Hazlett BA, Gherardi F (2002) Multimodal communication in crayfish: sex recognition during mate search by male *Austropotamobius pallipes*. Canadian Journal of Zoology 80: 2041–2045

Anger K, Moreira GS (1998) Morphometric and reproductive traits of tropical caridean shrimps. Journal of Crustacean Biology 18: 823–838

Antokhina TI, Britayev TA (2020) Host recognition behaviour and its specificity in pontoniine shrimp *Zenopontonia soror* (Nobili, 1904)(Decapoda: Caridea: Palaemonidae) associated with shallow-water sea stars. Journal of Experimental Marine Biology and Ecology 524: 151–302

Aoyagi M (2014) A new record of *Tachaea chinensis* Thielemann (Isopoda, Corallanidae) from Okinawa Island, Ryukyu Islands, Japan. Biol Mag Okinawa 52: 65–68 (in Japanese with English abstract)

Aquiloni L, Gherardi F (2008) Assessing mate size in the red swamp crayfish *Procambarus clarkii*: effects of visual versus chemical stimuli. Freshwater Biology 53: 461–469

Baeza JA, Stotz W (2003) Host-use and selection of differently colored sea anemones by the symbiotic crab *Allopetrolisthes spinifrons*. Journal of Experimental Marine Biology and Ecology 284: 25–39

Baillie C, Welicky RL, Hadfield KA, Smit NJ, Mariani SD, Beck RM (2019) Hooked on you: shape of attachment structures in cymothoid isopods reflects parasitic strategy. BMC Evolutionary Biology 19:1–11

Barber BJ, Blake NJ (2006) Reproductive physiology. Developments in aquaculture and fisheries science 35: 357–416

Bass CS, Weis JS (1999) Behavioral changes in the grass shrimp, *Palaemonetes pugio* (Holthuis), induced by the parasitic isopod, *Probopyrus pandalicola* (Packard). Journal of Experimental Marine Biology and Ecology 241:223–233

Beck JT (1980)a Life history relationships between the bopyrid isopod *Probopyrus pandalicola* and of its freshwater shrimp hosts *Palaemonetes paludosus*. American Midland Naturalist, 104: 135–154

Beck JT (1980)b The effects of an isopod castrator, *Probopyrus pandalicola*, on the sex characters of one of its caridean shrimp hosts, *Palaemonetes paludosus*. The Biological Bulletin, 158: 1–15

Bowman TE, Botosaneanu L (1992) First record of a *Tachaea* species from Sulawesi (Indonesia) with description of its manca stage (Isopoda, Flabellifera, Corallanidae). Bijdragen tot de Dierkunde 62: 111–115

Boxshall G, Hayes P (2019) Biodiversity and taxonomy of the parasitic Crustacea. Parasitic Crustacea 73–134

Bruce NL (2004) Crustacea: Isopoda. Freshwater invertebrates of the Malaysian region. Academy of Sciences Malaysia (ed. Yule K and Hoi Sen Y), Kuala Lumpur, pp 298–310

Bruce NL, Brusca RC, Delaney PM (1982) The status of the isopod families Corallanidae Hansen, 1890, and Excorallanidae Stebbing, 1904 (Flabellifera). Journal of Crustacean Biology 2:464-468.

Bunkley-Williams L, Williams EH (1998) Isopods associated with fishes: a synopsis and corrections. The Journal of parasitology 84: 893–896

Calado R, Bartilotti C, Narciso L (2005) Short report on the effect of a parasitic isopod on the reproductive performance of a shrimp. Journal of Experimental Marine Biology and Ecology 321: 13–18

Calado R, Vitorino A, Dinis MT (2006) Bopyrid isopods do not castrate the simultaneously hermaphroditic shrimp *Lysmata amboinensis* (Decapoda: Hippolytidae). Diseases of Aquatic Organisms 73: 73–76

Castro P (1988) Animal symbioses in coral reef communities: a review. Symbiosis 5:161–184

Chiussi R, Diaz H (2002) Orientation of the fiddler crab, *Uca cumulanta*: responses to chemical and visual cues. Journal of chemical ecology 28: 1787–1796

Coile AM, Sikkel PC (2013) An experimental field test of susceptibility to ectoparasitic gnathiid isopods among Caribbean reef fishes. Parasitology 140: 888–896

Cook C, Munguia P (2013) Sensory cues associated with host detection in a marine parasitic isopod. Marine Biology 160: 867–875

De Bruyn C, De Ridder C, Rigaud T, David B (2011) Chemical host detection and differential attraction in a parasitic pea crab infecting two echinoids. Journal of experimental marine biology and ecology 397: 173–178

Delaney PM (1989) Phylogeny and biogeography of the marine isopod family Corallanidae (Crustacea, Isopoda, Flabellifera). Natural History Museum of Los Angeles County 409: 1 – 75

Dumbauld BR, Chapman JW, Torchin ME, Kuris AM (2011) Is the collapse of mud shrimp (*Upogebia pugettensis*) populations along the Pacific coast of North America caused by outbreaks of a previously unknown bopyrid isopod parasite (*Orthione griffenis*)?. Estuaries and Coasts 34: 336–350

Felgenhauer BE (1992) Internal anatomy of the Decapoda: an overview. Microscopic anatomy of invertebrates 10: 45–75

Fogelman RM, Kuris AM, Grutter AS (2009) Parasitic castration of a vertebrate: effect of the cymothoid isopod, *Anilocra apogonae*, on the five-lined cardinalfish, *Cheilodipterus quinquelineatus*. International Journal for Parasitology 39: 577–583

Gentil-Vasconcelos HC, Tavares-Dias M (2015) First study on infestation of *Excorallana berbicensis* (Isopoda: Corallanidae) on six fishes in a reservoir in Brazilian Amazon during dry and rainy seasons. Lat Am J Aquat Res 43: 936 – 943

Gokoglu M, Teker S, Korun J (2020) Infestation rate and impacts of *Epipenaeon ingens* on growth and reproduction of brown shrimp (*Penaeus aztecus*). Acta Aquatica: Aquatic Sciences Journal 7: 50–53

Gopalakrishnan A, Raja K, Trilles JP, Rajkumar M, Rahman MM, Saravanakumar A (2017) Bopyrid isopods parasitizing on the cultured fresh water prawn, *Macrobrachium malcolmsonii* in South India. Journal of Parasitic Diseases 41: 93–96

Grave SD, Cai Y, Anker A (2007) Global diversity of shrimps (Crustacea: Decapoda: Caridea) in freshwater. Hydrobiology 595: 287 – 293

Grutter AS (1994) Spatial and temporal variations of the ectoparasites of seven reef fish species from Lizard Island and Heron Island, Australia. Marine Ecology Progress Series 115:21–30

Guzman HM, Obando VL, Brusca RC, Delaney PM (1988) Aspects of the population biology of the marine isopod Excorallana *Tricornis occidentalis* Richardson, 1905 (Crustacea: Isopoda: Corallanidae) at Cano Island, Pacific Costa Rica. Bulletin of Marine Science 43: 77–87

Hebets EA, Rundus A (2010) Chemical communication in a multimodal context. In Chemical communication in crustaceans. Springer, New York, pp 335–354

Ho JS, Tonguthai K (1992) Flabelliferan isopods (Crustacea) parasitic on freshwater fishes of Thailand. Systematic parasitology 21: 203–210

Hua CJ, Zhang D, Zou H, Wu SG, Wang GT, Braicovich PE, Li WX (2017) Effects of the parasitic isopod *Ichthyoxenus japonensis* Richardson, 1913 (Isopoda, Cymothoidae) on the growth and gonad development of the goldfish *Carassius auratus* Linnaeus, 1758. The Journal of Crustacean Biology 37: 356–358

Huang HD, Rittschof D, Jeng MS (2005) Visual orientation of the symbiotic snapping shrimp *Synalpheus demani*. Journal of Experimental Marine Biology and Ecology 326: 56–66

Imai T, Oonuki T (2014) Records of Chinese grass shrimp, *Palaemonetes sinensis* (Sollaud, 1911) from western Japan and simple differentiation method with native freshwater shrimp, *Palaemon paucidens* De Haan, 1844 using eye size and carapace color pattern. BioInvasions records 3: 163–168

Imai T, Ogasawara C, Saito H (2021) Additional records of freshwater shrimp, *Palaemon sinensis* (Sollaud, 1911) with a note on ectoparasitic isopod, *Tachaea chinensis* Thielemann, 1910 from Ehime Prefecture, Japan. Nanki Seibutsu 63: 67–72 (in Japanese)

Kamio M, Reidenbach MA, Derby CD (2008) To paddle or not: context dependent courtship display by male blue crabs, *Callinectes sapidus*. Journal of Experimental Biology 211: 1243–1248

Kawai T, Kobayashi Y (2005) Origin and current distribution of the alien crayfish, *Procambarus clarkii* (Girard, 1852) in Japan. Crustaceana 78: 1143–1149

Khalfan AWM, Imai T, Hashem S, Tani S, Saito H (2022) Size Relationship of the Ectoparasite *Tachaea chinensis* in the Host Shrimp *Palaemon paucidens* From Shiga Prefecture, Japan. Zoological Science 39: 336–342

Leung TL, Poulin R (2008) Parasitism, commensalism, and mutualism: exploring the many shades of symbioses. Vie et Milieu/Life & Environment 107–115

Li X, Chen Q, Li Y (2021) The morphology of the parasitic isopod *Tachaea chinensis* (Isopoda, Cymothoida) revealed through scanning electron microscopy and histological analysis. Crustaceana 94: 63–75

Li Y, Han Z, Xu W, Li X, Zhao Y, Wei H, Li X, Chen Q (2020) Antioxidant and immune responses of the Oriental river prawn *Macrobrachium nipponense* to the isopod parasite *Tachaea chinensis*. Fish & shellfish immunology 101: 78–87

Li Y, Li X, Han Z, Xu W, Li X, Chen Q (2019)a Comparative tandem mass tag-based quantitative proteomic analysis of *Tachaea chinensis* isopod during parasitism. Frontiers in cellular and infection microbiology 9: 350

Li Y, Li X, Xu W, Han Z, Zhao Y, Dong J, Wei H, Chen Q (2019)b Comparative iTRAQ-based quantitative proteomic analysis of the Chinese grass shrimp (*Palaemonetes sinensis*) infected with the isopod parasite *Tachaea chinensis*. Parasites & vectors 12: 1–12

Li Y, Xu W, Li X, Jiang H, She Q, Han Z, Li X, Chen Q (2018) Comparative transcriptome analysis of Chinese grass shrimp (*Palaemonetes sinensis*) infected with isopod parasite *Tachaea chinensis*. Fish Shellfish Immunol 82: 153–161

Limviriyakul P, Tseng LC, Shih TW, Hwang JS (2016) Host selection and preferences of coral symbiotic crab *Tetralia rubridactyla*. Journal of Experimental Marine Biology and Ecology 485: 24–34

Lozano GA (1991) Optimal foraging theory: a possible role for parasites. Oikos 60: 391-395

Machida Y, Akiyama YB (2013) Impacts of invasive crayfish (*Pacifastacus leniusculus*) on endangered freshwater pearl mussels (*Margaritifera laevis* and *M. togakushiensis*) in Japan. Hydrobiologia 720: 145–151

Mancinelli G (2010) Intraspecific, size-dependent variation in the movement behaviour of a brackish-water isopod: a resource-free laboratory experiment. Marine and Freshwater Behaviour and Physiology 43: 321–337

March JG, Pringle CM, Townsend MJ, Wilson AI (2002) Effects of freshwater shrimp assemblages on benthic communities along an altitudinal gradient of a tropical island stream. Freshwater Biology 47: 377–390

Mariappan P, Balasundaram C, Trilles JP (2003) Infection of the isopod *Tachaea spongillicola* on freshwater prawns *Macrobrachium* spp. in southern India. Dis Aquat Org 55: 259–260

Nagasawa K, Imai T, Saito H (2018) *Tachaea chinensis* (Isopoda: Corallanidae), an ectoparasite of freshwater shrimps and prawns, from western Japan, with a list of its known collection localities and hosts. Crustacean research 47: 73–88

Nagel L, Grutter AS (2007) Host preference and specialization in Gnathia sp., a common parasitic isopod of coral reef fishes. Journal of Fish Biology 70: 497–508

Nagler C, Hyžný M, Haug JT (2017) 168 million years old "marine lice" and the evolution of parasitism within isopods. BMC Evolutionary Biology 17: 1–14

Nakachi Y, Shimizu T, Nogami N, Makita S (2010) Studies on *Tachaea chinensis* II. Blood-sucking behavior of *T. chinensis*. Umiushi Tsushin 66: 10 (in Japanese)

Nishino M (1980) Geographical Variations in Body Size, Brood Size and Egg Size of a Freshwater Shrimp, *Palaemon paucidens* DE HAAN, with Some Discussionon Brood Habit. Japanese Journal of Limnology (Rikusuigaku Zasshi) 41: 185–202

Niwa N (2010) Invasion and dispersion routes of alien alive freshwater shrimps *Neocaridina* spp. (Caridea, Atyidae) and Palaemonidae spp. (Caridea), imported into Japan. Cancer 19: 75–80

Nunomura N (2006) A new species of the genus *Tachaea* (Isopoda, Corallanidae) from the Lake Tonle Sap, Cambodia. Bull Toyama Sci Mus 29: 17–21

Nunomura N, Shimomura M (2020) Isopoda from Japan (61) Suborder Cymothoidea, Family Corallanidae, genera *Tachaea*, *Excorallana*, *Argathona*, *Alcirona*. Aquabiology 42: 87–93 (in Japanese with English abstract)

Ogasawara C, Imai T, Kodama A, Fatsi PSK, Hashem S, Appiah EK, Tettey PA, Saito H (2021) Population genetics of the non-native freshwater shrimp *Palaemon sinensis* (Sollaud, 1911) in Japan based on mitochondrial 16S rRNA sequence analysis. Aquatic Invasions 10: 710–720

Ogawa Y, Kakuda S (1988) Mating and Hatching Behaviour of the lake prawn *Palaemon paucidens* De Haan (Decapoda, Palaemonidae). Aquaculture Science 36: 151–156

Olabarria C, Underwood A, Chapman M (2002) Appropriate experimental design to evaluate preferences for microhabitat: an example of preferences by species of microgastropods. Oecologia 132: 159–166

Oonuki T, Suzuki N, Akiyama N (2010) Annual reproductive cycle of the female *Palaemonetes sinensis* recorded for the first time in a pond of Hamamatsu City, Shizuoka Prefecture, Japan. Aquacult Sci 58: 509–516 (in Japanese with English abstract)

Ory NC, Thiel M (2013) Host-use patterns and factors influencing the choice between anemone and urchin hosts by a caridean shrimp. Journal of experimental marine biology and ecology 449: 85–92

Ota Y (2013) Host utilization in gnathiid isopod, temporal ectoparasite of fishes. Cancer 22:57–63 (in Japanese)

Ota Y (2019) Habitat utilization and seasonal occurrence of *Tachaea chinensis* (Isopoda: Corallanidae) infesting freshwater shrimps in Lake Biwa, central Japan. Crustacean research 48: 133–143

Pawluk RJ, Ciampoli M, Mariani S (2014) Host size constrains growth patterns in both female and male *Ceratothoa italica*, a mouth-dwelling isopod. Marine and Freshwater Research 66: 381–384

Penfold R, Grutter AS, Kuris AM, McCormick MI, Jones CM (2008) Interactions between juvenile marine fish and gnathiid isopods: predation versus micropredation. Marine Ecology Progress Series 357:111–119

Petrić M, Ferri J, Mladineo I (2010) Growth and reproduction of *Munida rutllanti* (Decapoda: Anomura: Galatheidae) and impact of parasitism by *Pleurocrypta* sp.(Isopoda: Bopyridae) in the Adriatic Sea. Journal of the Marine Biological Association of the United Kingdom 90: 1395–1404

Poulin R (1995) Evolutionary influences on body size in free-living and parasitic isopods. Biological Journal of the Linnean Society 54: 231–244

Pralon BGN, Antunes M, Mortari RC, Bueno SLDS, Negreiros-Fransozo ML (2018) Infestation of two shrimp species of the genus *Palaemon* Fabricius, 1798 (Decapoda, Palaemonidae) by an isopod of the genus *Probopyrus* Giard & Bonnier, 1888 (Bopyridae) from the Brazilian southeast coast. Nauplius 26: 1–8

Puspitasari I, Oh CW, Lee WO (2013) Reproductive traits of the lake prawn *Palaemon paucidens* De Haan, 1844 (Decapoda, Palaemonidae), from Goesan lake, South Korea. Crustaceana 86: 1191–1205

Rameshkumar G, Ravichandran S (2013) Histopathological changes in the skins and gills of some marine fishes due to parasitic isopod infestation. Journal of Coastal Life Medicine 1: 12–18

Rameshkumar G, Ravichandran S (2014) Problems caused by isopod parasites in commercial fishes. Journal of Parasitic Diseases 38: 138–141

Riek EF (1967) A new corallanid isopod parasitic on Australian freshwater prawns. In Proceedings of the Linnean Society of New South Wales. 91: 176 –178

Rochet MJ (2000) May life history traits be used as indices of population viability?. Journal of Sea Research 44: 145–157

Rosenberg E, Zilber-Rosenberg I (2011) Symbiosis and development: the hologenome concept. Birth Defects Research Part C: Embryo Today: Reviews 93: 56–66

Sahoo AK, Sadhukhan D, Meena DK, Das T, Raman RK, Udgata SK, Shashikala KB, Mitra S, Das BK (2022) Wild fish of aquaculture importance, as new host for isopod *Tachaea spongillicola* Stebbing, 1907 infestation with emphasis on prevalence and histopathology: A case report. Aquaculture 547: 737436

Saito H (2017) Occurrence of the exotic freshwater shrimp *Palaemon sinensis* in central Hiroshima Prefecture, Japan. Fisheries Science 83: 837–843

Saito H (2018) Effect of newly established import quarantine regulations on the supply of alien palaemonid shrimp *Palaemon sinensis* in Japan. Nippon Suisan Gakkaishi 84: 87–93 (in Japanese with English abstract)

Saito H, Kometani K, Kodama A (2019) Seasonal occurrence of the alien freshwater shrimp *Palaemon sinensis* (Sollaud, 1911) in lower reaches of a river in western Japan. BioInvasions Rec 8: 369–378

Saito H, Niwa N, Kawai K, Imabayashi H (2011) Current state of aquatic animals sold as sport fishing bait in Western Japan. Bulletin of the Hiroshima University Museum 3: 45–57

Saito H, Yamasaki A, Watanabe J, Kawai K (2016) Distribution of the invasive freshwater shrimp *Palaemon sinensis* (Sollaud, 1911) in rivers of Hiroshima Prefecture, western Japan. BioInvasions Rec 5: 93–100

Saito N (2011) Parasitic crustaceans found on freshwater decapods. In "Shrimps, Crabs, and Crawfishes—Conservation and Biology of Freshwater Crustaceans" Ed by T Kawai, K Nakata, Seibutsu Kenkyusha, Tokyo, pp 173–175 (in Japanese)

Sehr EK, Gall BG (2016) Responses of an aquatic isopod and amphipod to chemical alarm cues from damaged conspecifics. Journal of Freshwater Ecology, 31: 231–237

Shen C-J (1936) The fresh-water isopods of Peiping. Bulletin of the Fan Memorial Institute of Biology (Zoology) 7: 1–31

Shiino S (1965) *Tachea* [sic] *chinensis* Thielemann. In "New Illustrated Encyclopedia of the Fauna of Japan (II)" Ed by Y Okada, S Uchida, T Uchida, Hokuryukan, Tokyo, p 542 (in Japanese)

Sikkel PC, Welicky R L (2019) The ecological significance of parasitic crustaceans. In Parasitic Crustacea (ed. N. Smit, N. Bruce and K. Hadfield) Springer, Cham, pp 421–477

Song J-H, Min G-S (2018) First records of *Gnathia* Leach, 1814 and *Tachaea* Schioedte & Meinert, 1879 from South Korea, with descriptions of two new species (Isopoda, Cymothoida, Cymothooidea). ZooKeys 787: 17–35

Souty-Grosset C, Anastacio PM, Aquiloni L, Banha F, Choquer J, Chucholl C, Tricarico E (2016) The red swamp crayfish *Procambarus clarkii* in Europe: impacts on aquatic ecosystems and human well-being. Limnologica, 58, 78–93

Stebbing TRR (1907) A freshwater isopod from Calcutta. Journal of the Linnean Society of London, Zoology 30: 39–42

Takahashi J (2015) Parasitization rate and its seasonal fluctuation of *Tachea* [sic] *chinensis* in *Paraemon* [sic] *pausidens*. Animate 12: 20–23 (in Japanese)

Tanaka K (2007) Life history of gnathiid isopods-current knowledge and future directions. Plankton and Benthos Research 2: 1–11

Thompson JN (1994) The coevolutionary process. University of Chicago press, pp 376

Tokaji H, Nakahara K, Goshima S (2014) Host switching improves survival rate of the symbiotic polychaete *Arctonoe vittata*. Plankton and Benthos Research 9:189–196

Toyota K, Seki S (2014) Freshwater Shrimps and Crabs of Japan: 102 Species from Fresh and Brackish Waters, Seibundo Shinokosha, Tokyo (in Japanese)

Tsai ML, Li JJ, Dai CF (2001) How host size may constrain the evolution of parasite body size and clutch size. The parasitic isopod *Ichthyoxenus fushanensis* and its host fish, *Varicorhinus bacbatulus*, as an example. Oikos, 92: 13–19

Ueki N, Sugiyama T, Muroga K (1988) Vibrio Infection in the Freshwater Shrimp (*Palaemon paucidence*) predisposed by a Parasitic Isopod (*Tachea chinensis*) Infestation. Fish Pathology 23: 175–178 (in Japanese with English abstract)

Welicky RL, Malherbe W, Hadfield KA, Smit NJ (2019) Understanding growth relationships of African cymothoid fish parasitic isopods using specimens from museum and field collections. International Journal for Parasitology: Parasites and Wildlife 8: 182–187

West LE, Randy Brooks W (2018) Host location and selection by the symbiotic sargassum crab *Portunus sayi*: the role of chemical, visual and tactile cues. Symbiosis 76: 177–185

Wilson GD (1991) Functional Morphology and Evolution of Isopod Genitalia. In Crustacean sexual biology (Eds. Bauer RT and Martin JW), Columbia University Press, pp 228–245

Wilson GD (2007) Global diversity of Isopod crustaceans (Crustacea; Isopoda) in freshwater. In Freshwater animal diversity assessment, Springer, Dordrecht, pp 231 –240

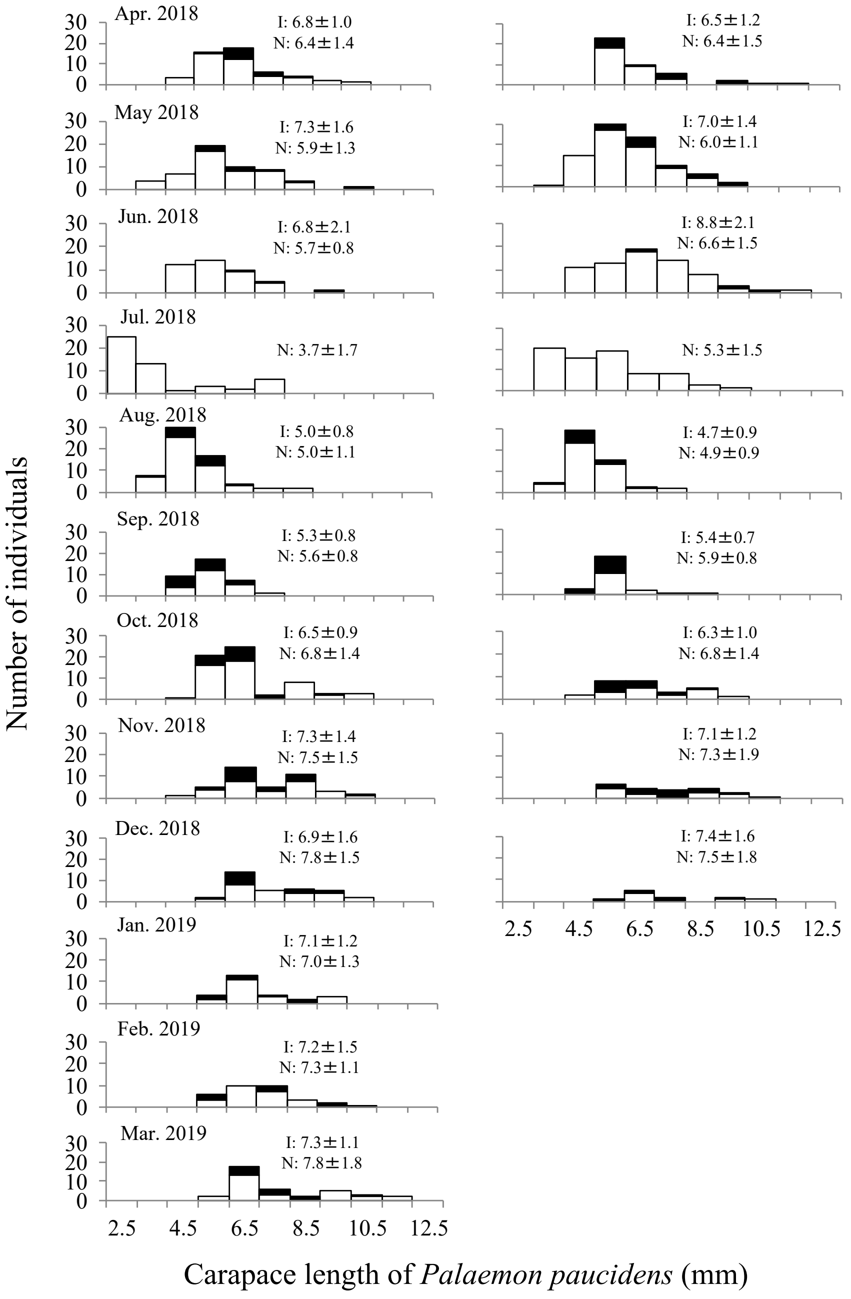
Xu W, Han Z, Xing Y, Li X, Zhao Y, Chen Q, Li Y (2019) Distribution of the parasitic isopod *Tachaea chinensis* in China. Scientific Reports 9: 1–7

Xu W, Li X, Han Z, Jin J, Zhao X, Zhao Y, Wei H, Chen Q, Li Y (2021) Life cycle of the ectoparasite *Tachaea chinensis* (Isopoda: Corallanidae) on the freshwater shrimp

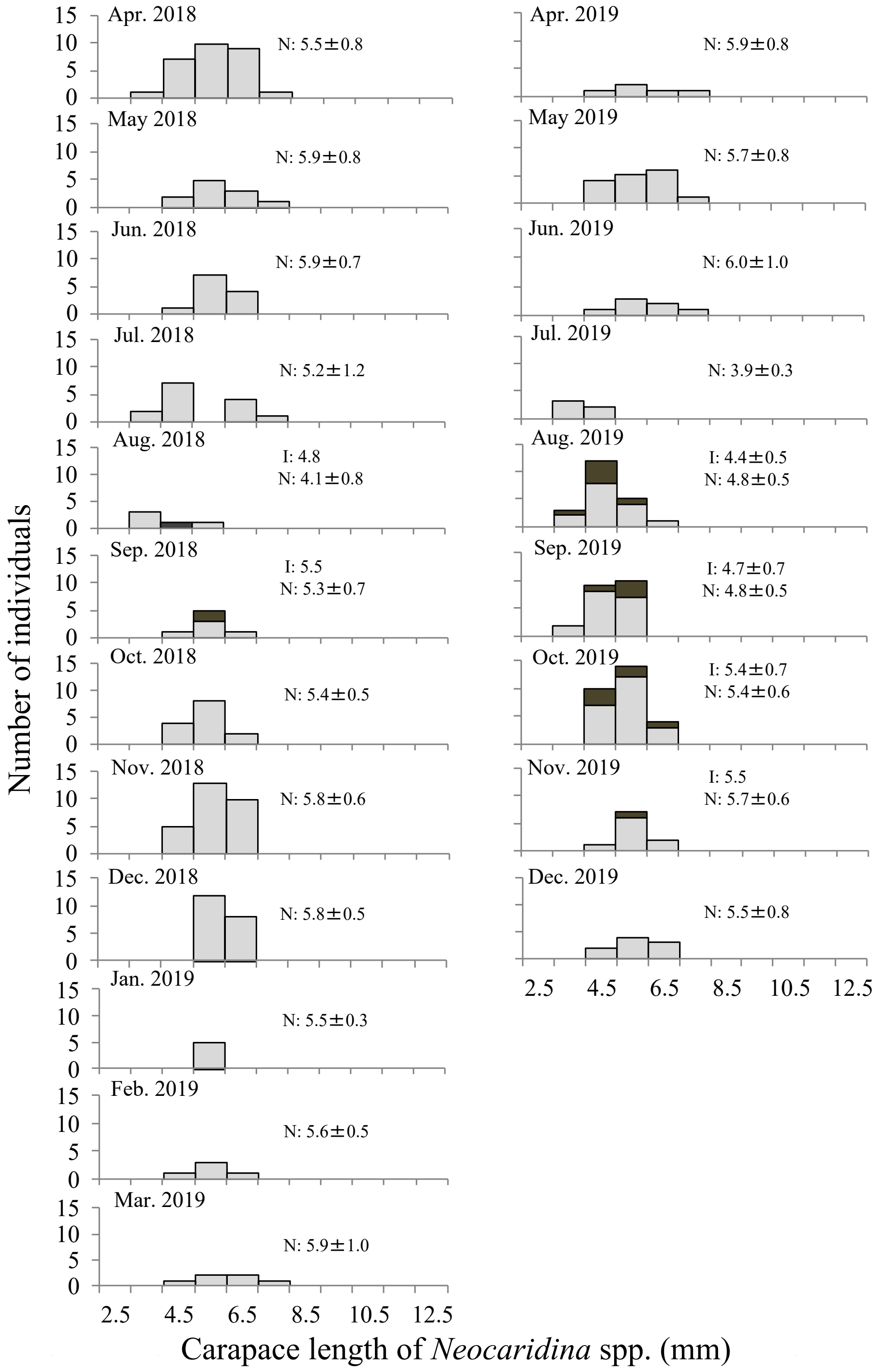
Palaemonetes sinensis (Decapoda: Palaemonidae). Diseases of aquatic organisms 144: 143–150

Yu C, Xu W, Li X, Jin J, Zhao X, Wang S, Zhang Z, Wei Y, Chen Q, Li Y (2021) Comparative transcriptome analysis of Chinese grass shrimp (*Palaemonetes sinensis*) hepatopancreas under ectoparasitic isopod (*Tachaea chinensis*) infection. Fish & Shellfish Immunology 117: 211–219

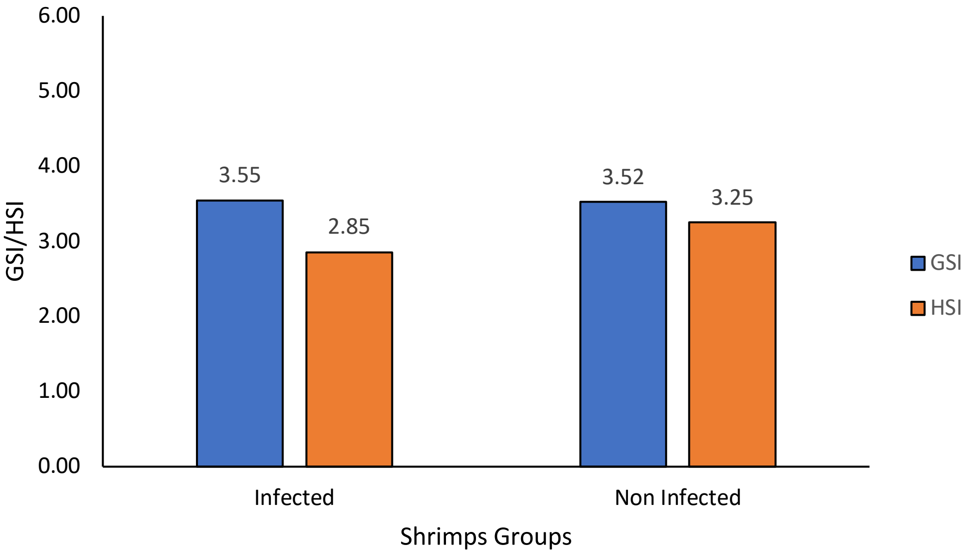
Appendices



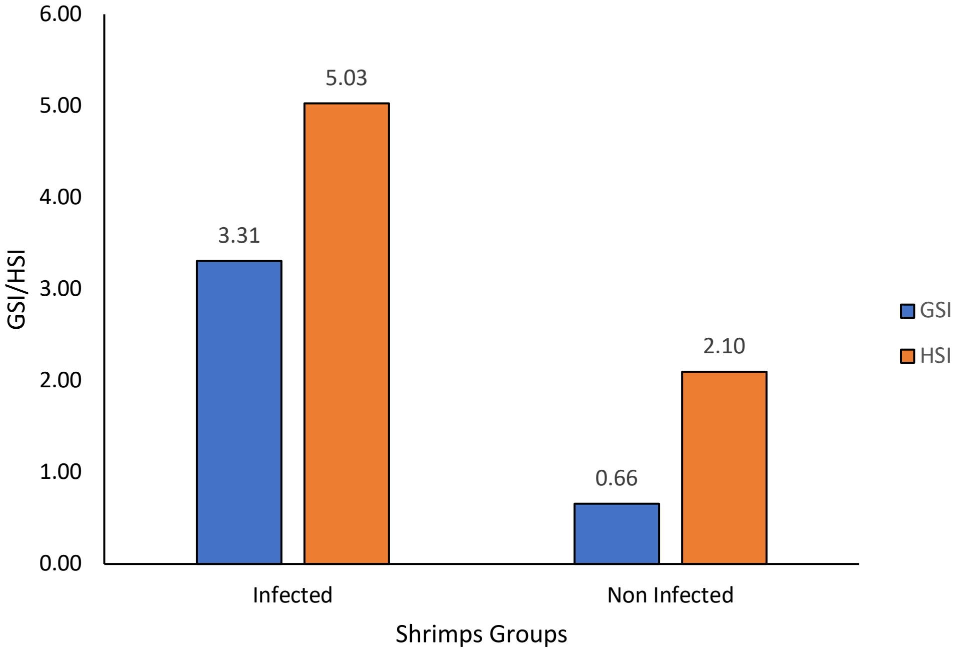
Appendix Figure S1: Size frequency distribution of the infested and uninfested *Palaemon paucidens* collected during the study. Values are expressed as mean ± standard deviation. *Mann-Whitney U-test, $P < 0.05$.



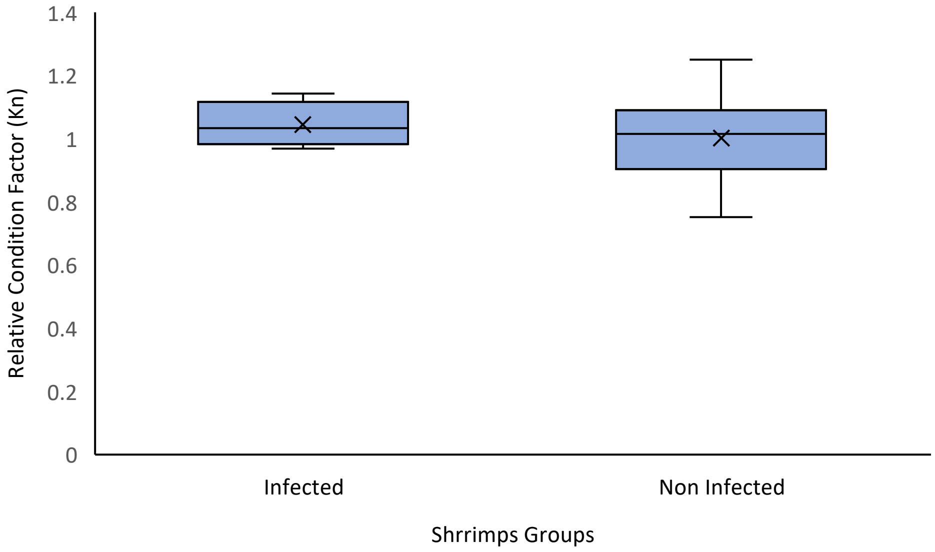
Appendix Figure S2: Size frequency distribution of the infested and uninfested *Neocaridina* spp. collected during the study. Values are expressed as mean ± standard deviation.



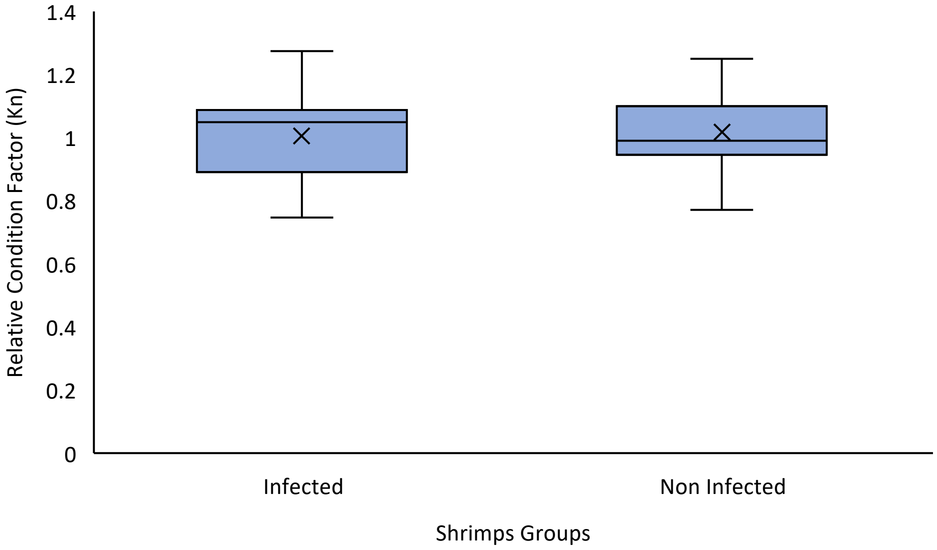
Appendix Figure S3: The average Gonadosomatic indexes (GSI) and Hepatosomatic indexes (HSI) of infected and non-infected females *Palaemon paucidens* collected from Okayama prefecture (n = 22).



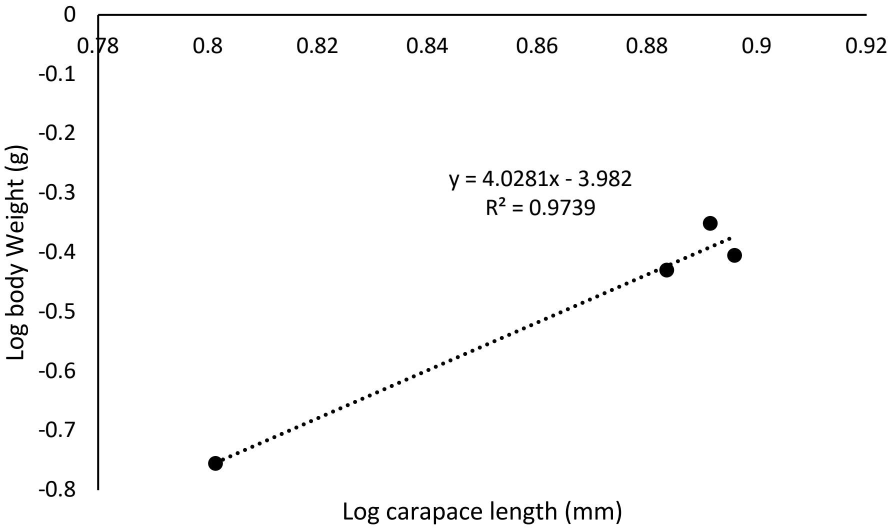
Appendix Figure S4: The average Gonadosomatic indexes (GSI) and Hepatosomatic indexes (HSI) of infected and non-infected males *Palaemon paucidens* collected from Okayama prefecture (n = 26).



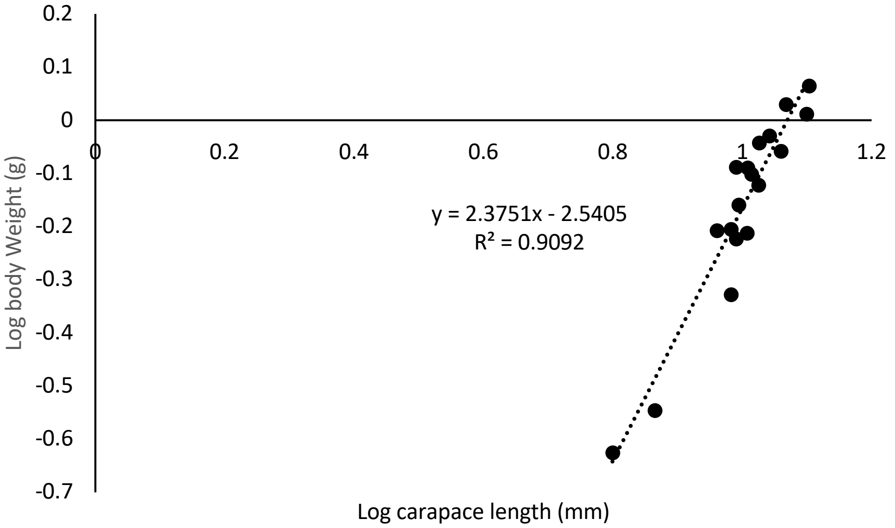
Appendix Figure S5: Boxplot of the relative condition factor (K_n) for infected and non-infected females *Palaemon paucidens* collected from Okayama prefecture. Boxplots are combined with a mean, minimum, maximum, first quartile, median and third quartile values.



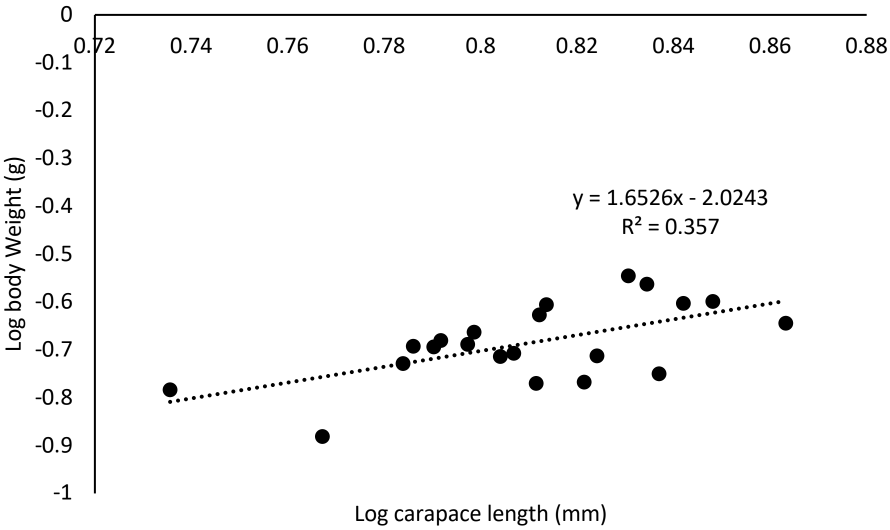
Appendix Figure S6: Boxplot of the relative condition factor (K_n) for infected and non-infected males *Palaemon paucidens* collected from Okayama prefecture. Boxplots are combined with a mean, minimum, maximum, first quartile, median and third quartile values.



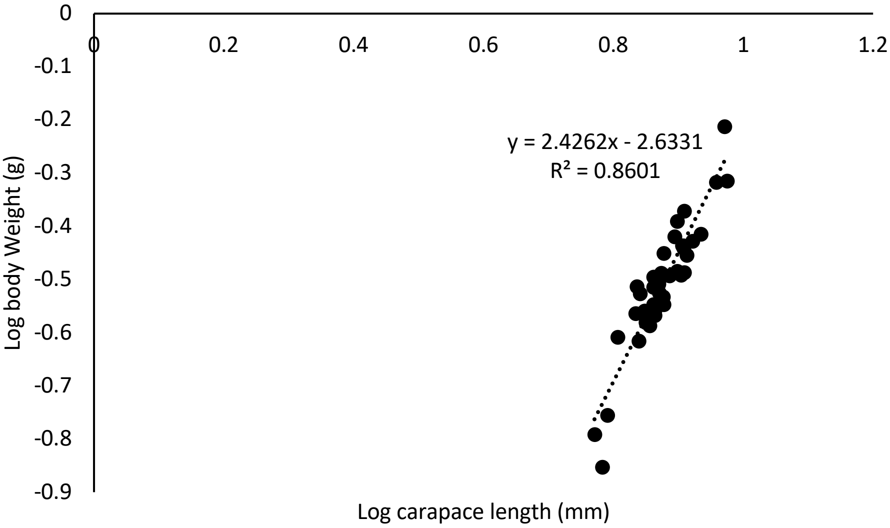
Appendix Figure S7: Carapace length- weight relationship of Infected females collected from Okayama prefecture.



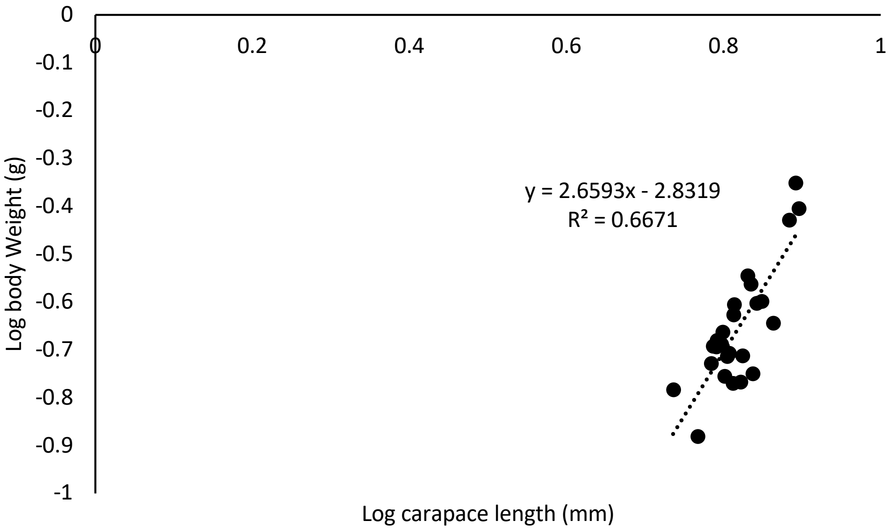
Appendix Figure S8: Carapace length- weight relationship of non-Infected females collected from Okayama prefecture.



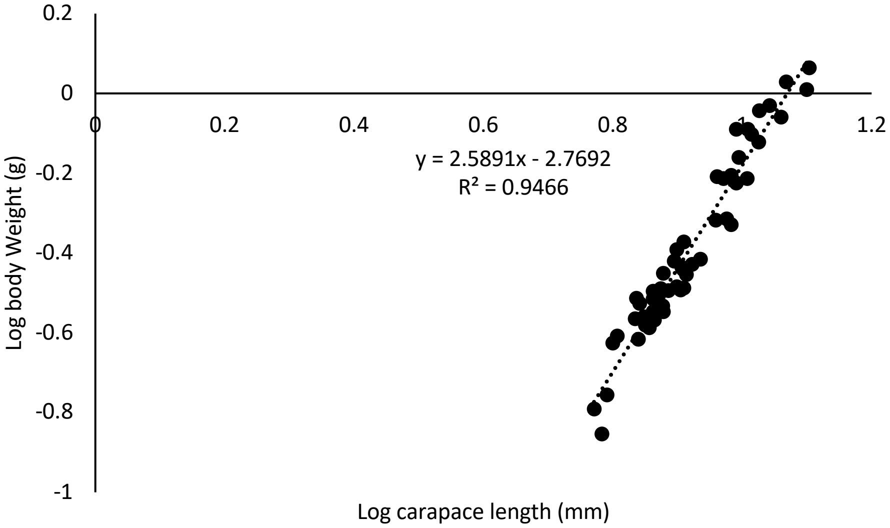
Appendix Figure S9: Carapace length- weight relationship of Infected males collected from Okayama prefecture.



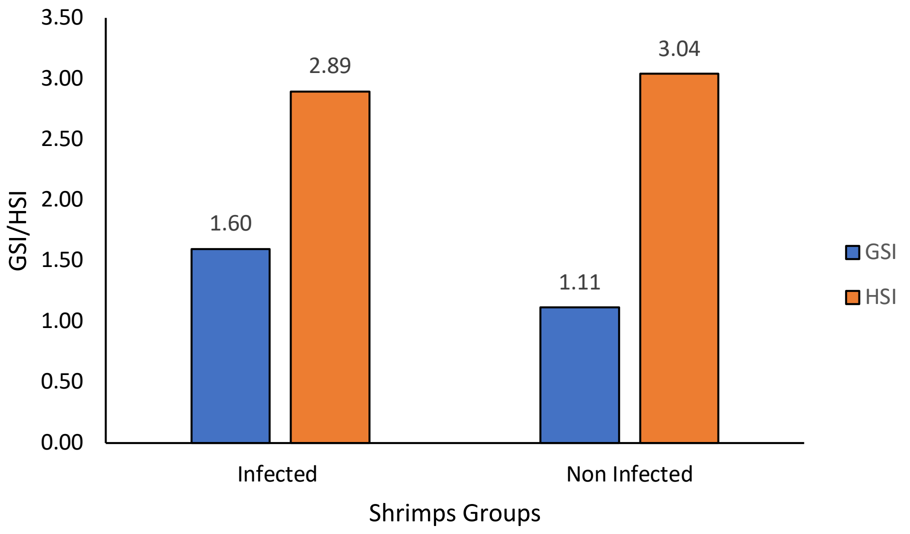
Appendix Figure S10: Carapace length- weight relationship of non-Infected males collected from Okayama prefecture.



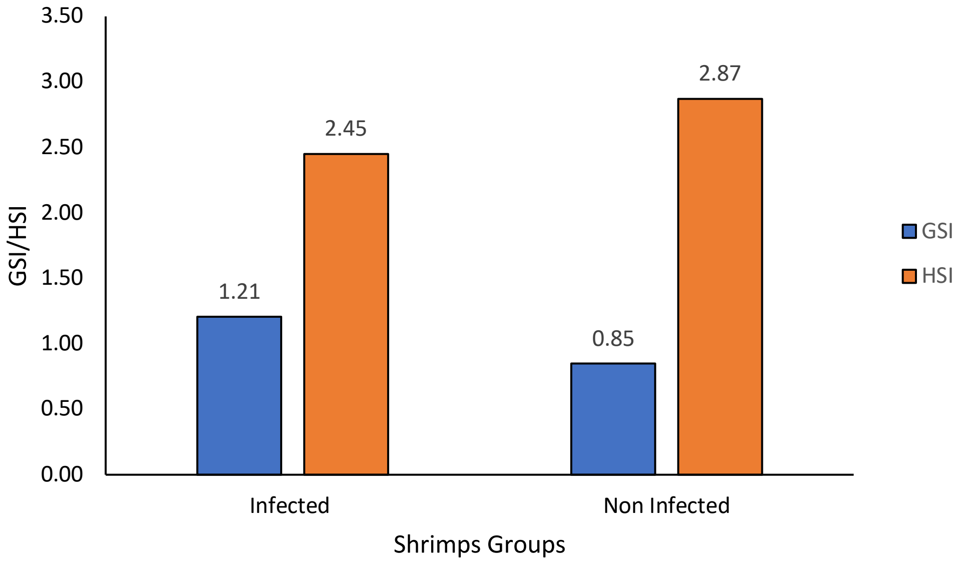
Appendix Figure S11: Carapace length- weight relationship of all Infected samples collected from Okayama prefecture.



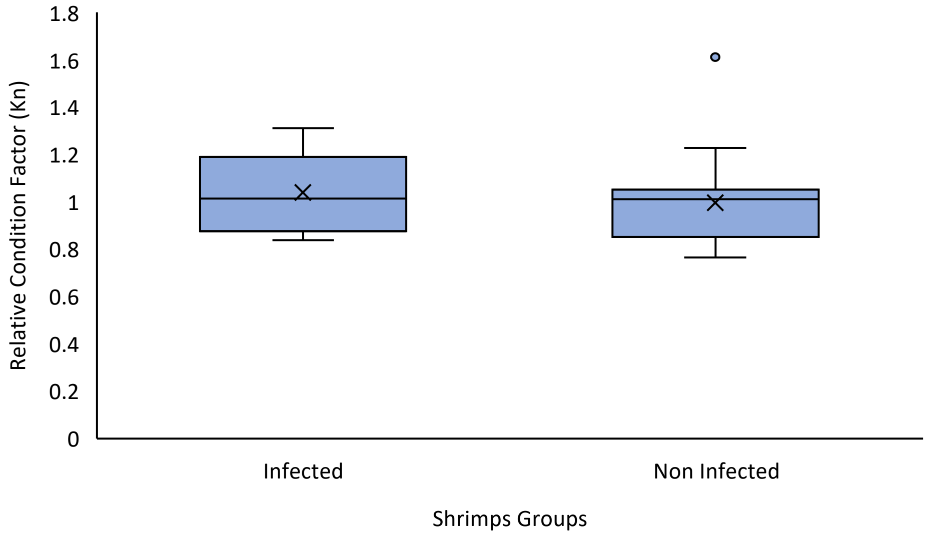
Appendix Figure S12: Carapace length- weight relationship of all non-Infected samples collected from Okayama prefecture.



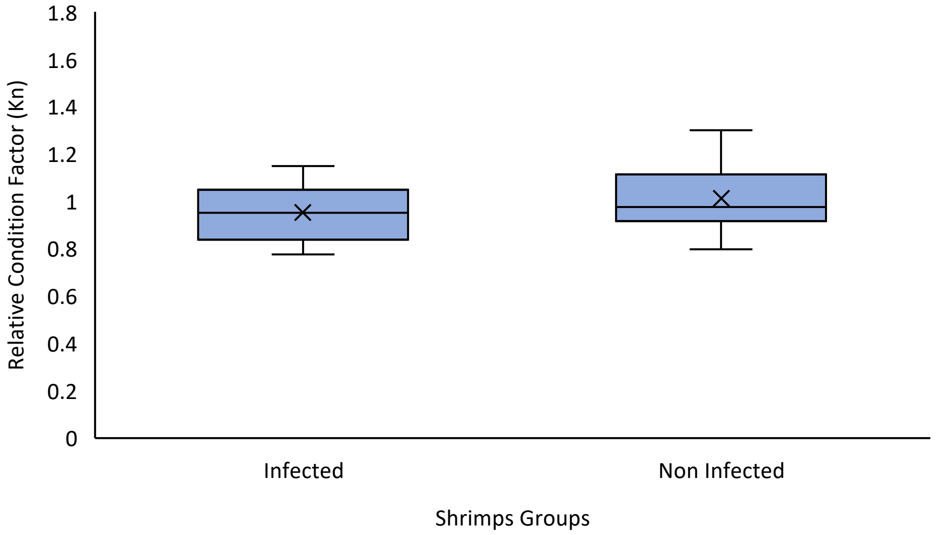
Appendix Figure S13: The average Gonadosomatic indexes (GSI) and Hepatosomatic indexes (HSI) of infected and non-infected females *Palaemon paucidens* collected from Shimane prefecture (n = 34).



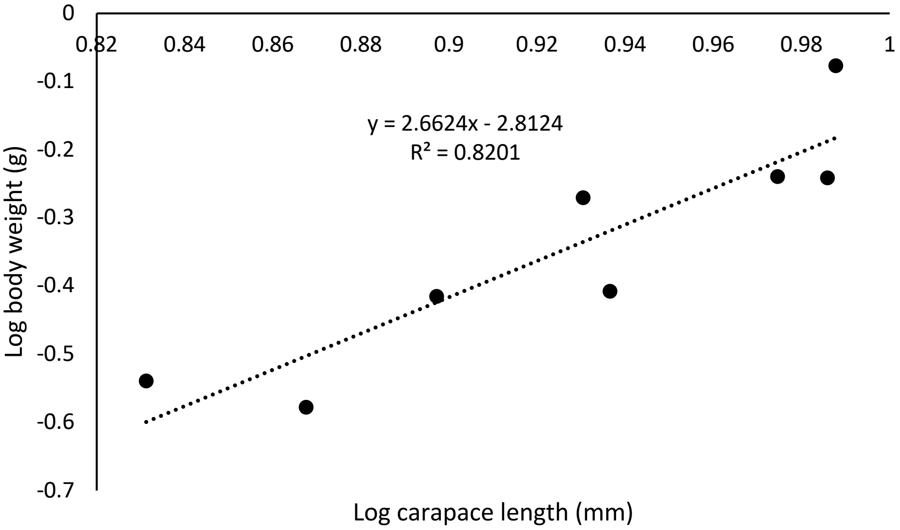
Appendix Figure S14: The average Gonadosomatic indexes (GSI) and Hepatosomatic indexes (HSI) of infected and non-infected males *Palaemon paucidens* collected from Shimane prefecture (n = 25).



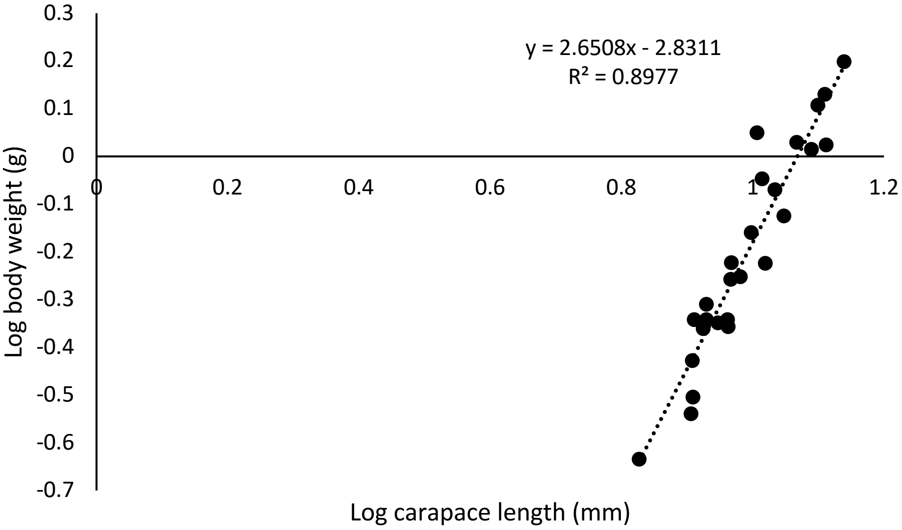
Appendix Figure S15: Boxplot of the relative condition factor (K_n) for infected and non-infected females *Palaemon paucidens* collected from Shimane prefecture. Boxplots are combined with a mean, minimum, maximum, first quartile, median and third quartile values.



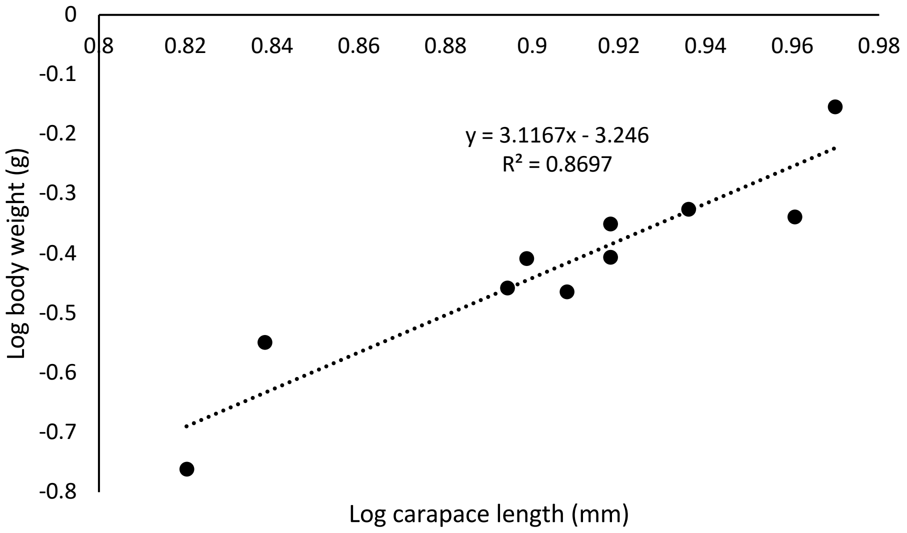
Appendix Figure S16: Boxplot of the relative condition factor (K_n) for infected and non-infected males *Palaemon paucidens* collected from Shimane prefecture. Boxplots are combined with a mean, minimum, maximum, first quartile, median and third quartile values.



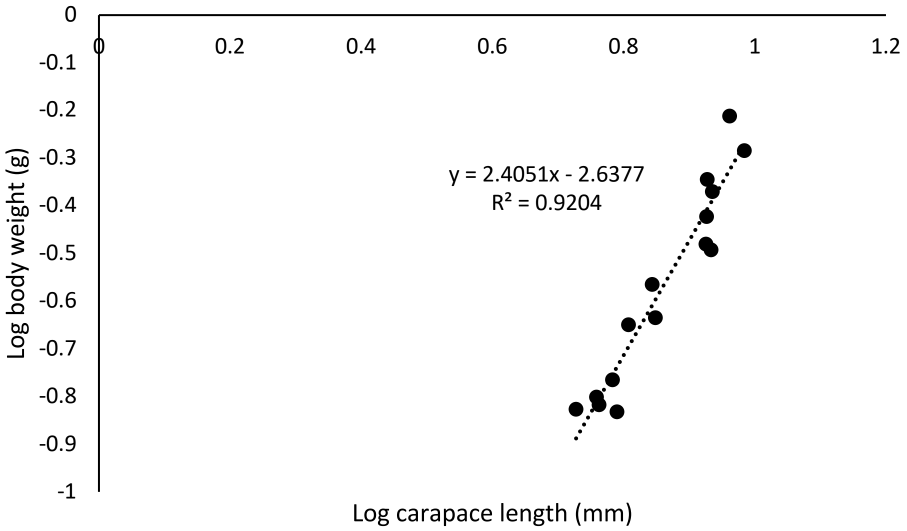
Appendix Figure S17: Carapace length- weight relationship of all Infected females collected from Shimane prefecture.



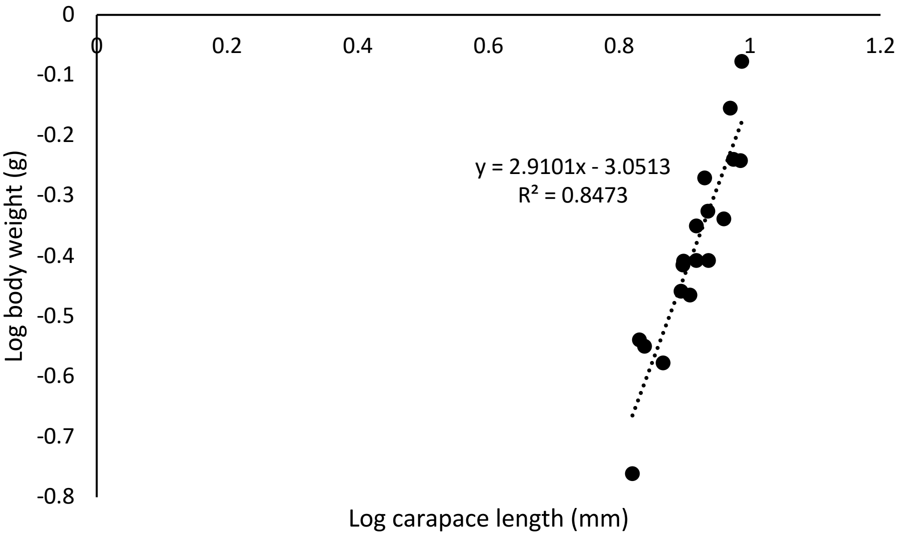
Appendix Figure S18: Carapace length- weight relationship of all non-Infected females collected from Shimane prefecture.



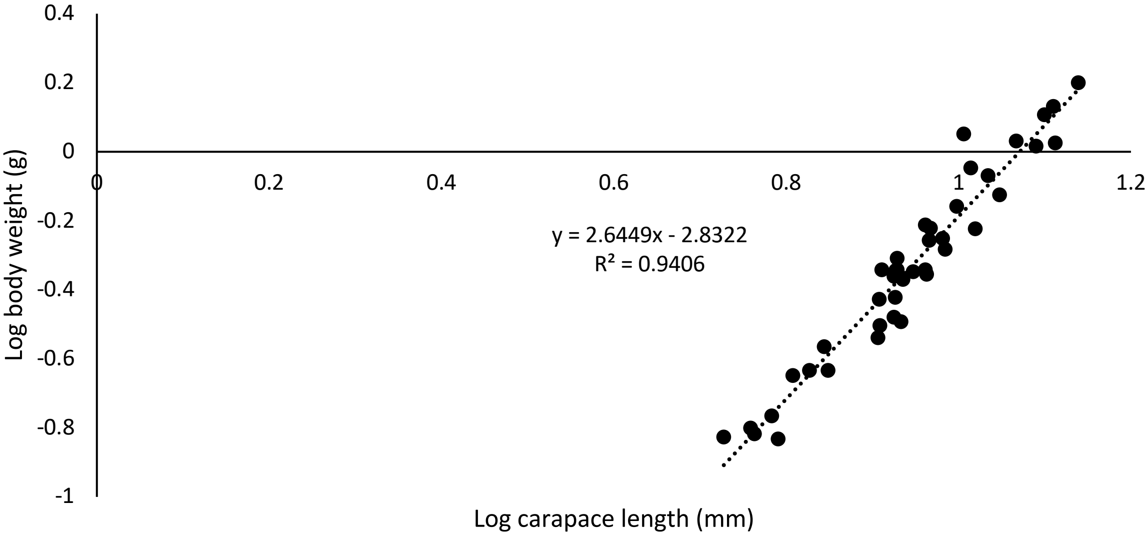
Appendix Figure S19: Carapace length- weight relationship of all Infected males *Palaemon paucidens* collected from Shimane prefecture.



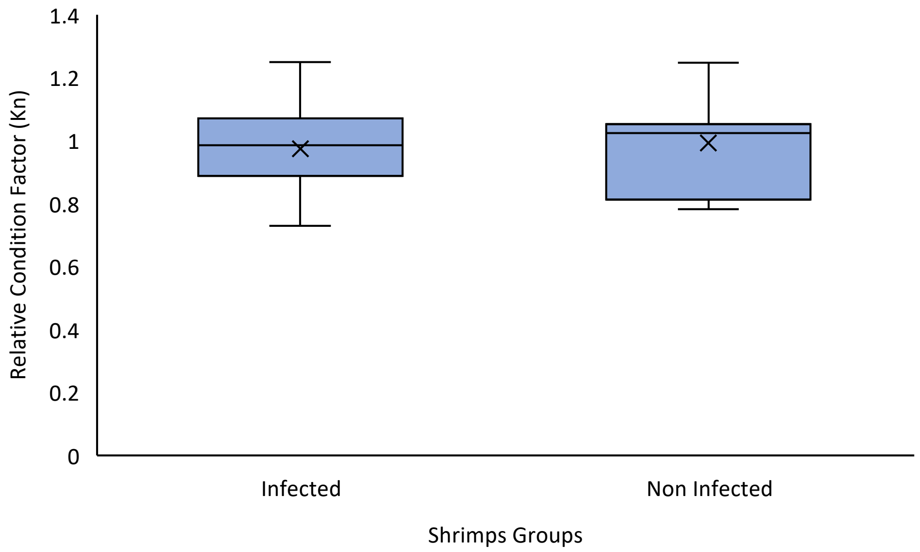
Appendix Figure S20: Carapace length- weight relationship of all non-Infected males *Palaemon paucidens* collected from Shimane prefecture.



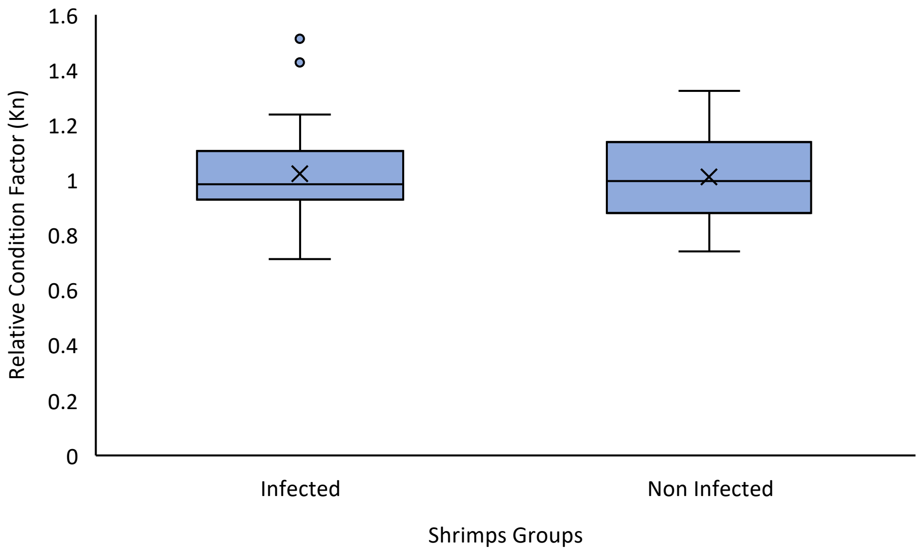
Appendix Figure S21: Carapace length- weight relationship of all Infected *Palaemon paucidens* collected from Shimane prefecture.



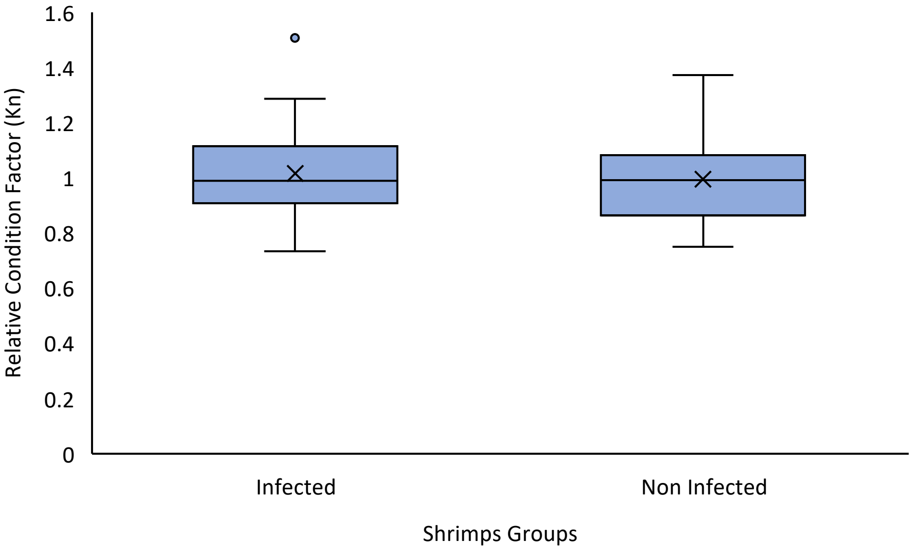
Appendix Figure S22: Carapace length- weight relationship of all non-Infected *Palaemon paucidens* collected from Shimane prefecture.



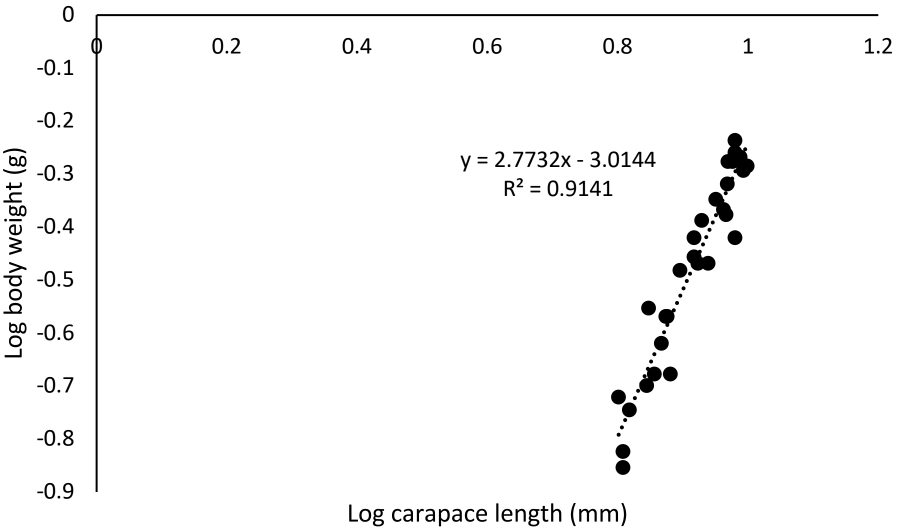
Appendix Figure S23: Boxplot of the relative condition factor (K_n) for infected and non-infected females *Palaemon paucidens* collected from Shimane prefecture during April 2018. Boxplots are combined with a mean, minimum, maximum, first quartile, median and third quartile values.



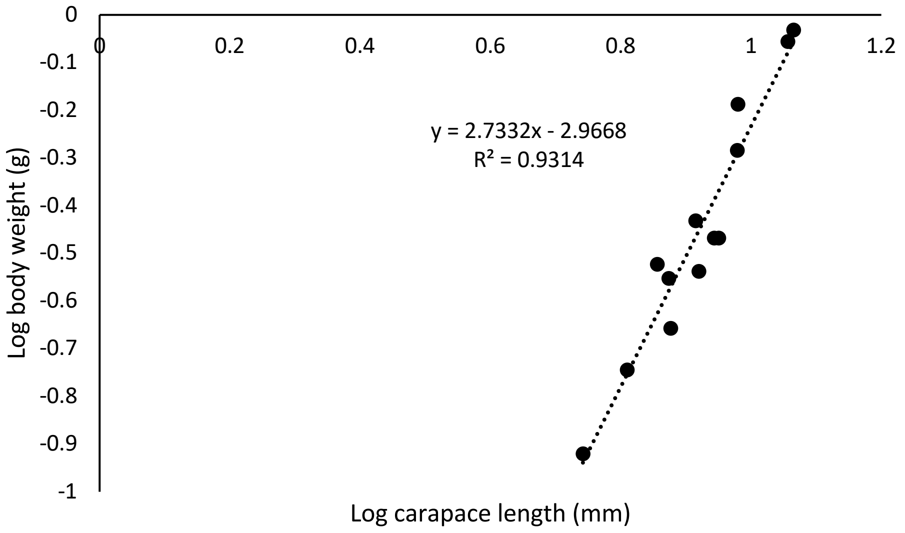
Appendix Figure S24: Boxplot of the relative condition factor (K_n) for infected and non-infected males *Palaemon paucidens* collected from Shimane prefecture during April 2018. Boxplots are combined with a mean, minimum, maximum, first quartile, median and third quartile values.



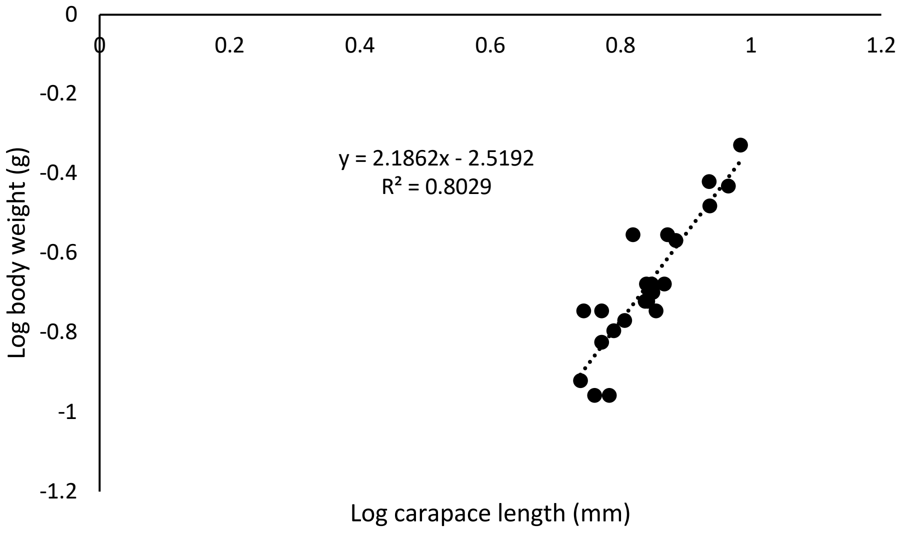
Appendix Figure S25: Boxplot of the relative condition factor (K_n) for infected and non-infected *Palaemon paucidens* collected from Shimane prefecture during April 2018. Boxplots are combined with a mean, minimum, maximum, first quartile, median and third quartile values.



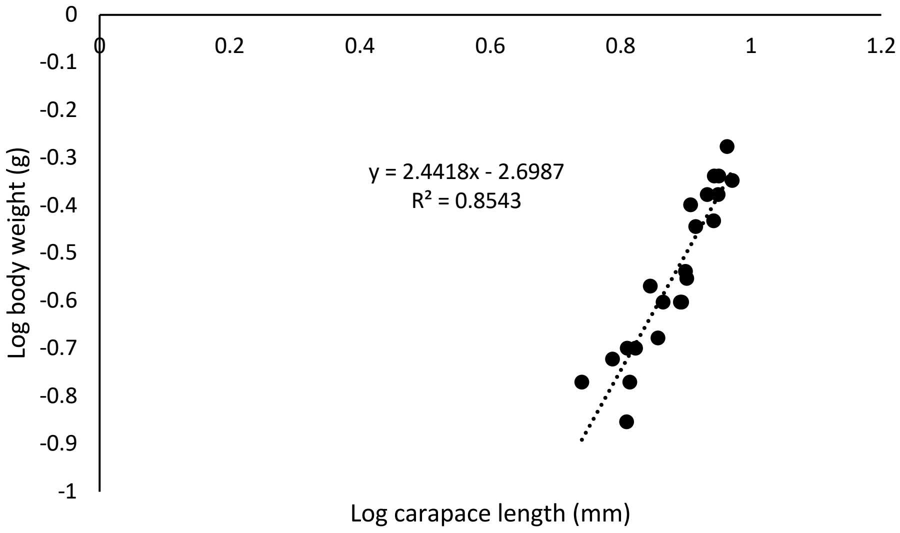
Appendix Figure S26: Carapace length- weight relationship of all Infected females *Palaemon paucidens* collected from Shimane prefecture during April 2018.



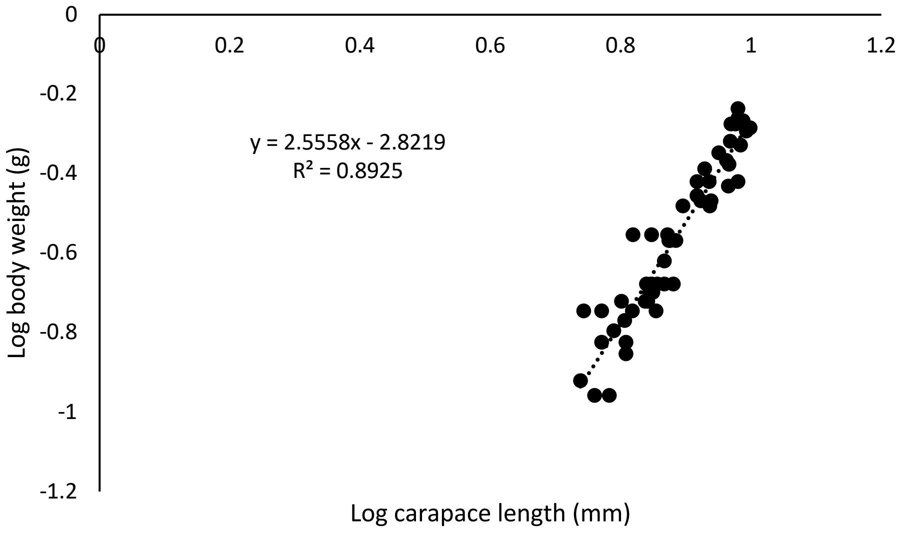
Appendix Figure S27: Carapace length- weight relationship of all non-Infected females *Palaemon paucidens* collected from Shimane prefecture during April 2018.



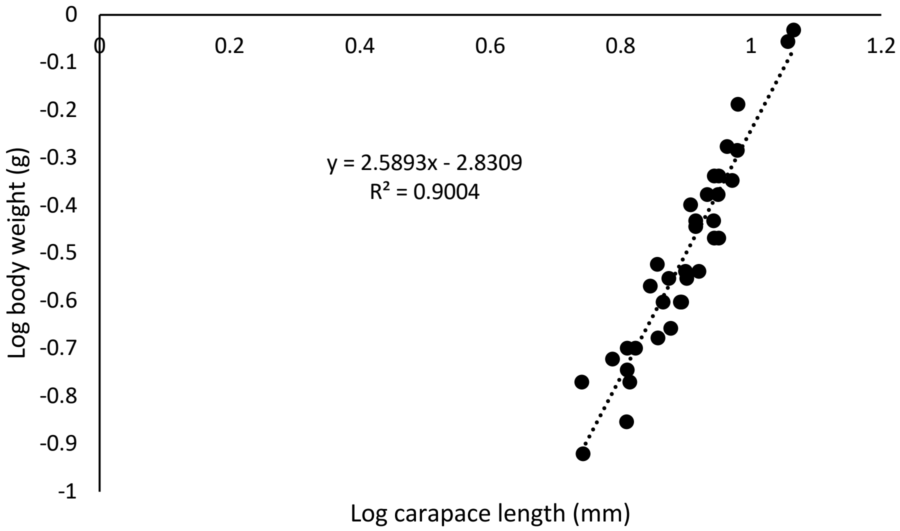
Appendix Figure S28: Carapace length- weight relationship of all Infected males *Palaemon paucidens* collected from Shimane prefecture during April 2018.



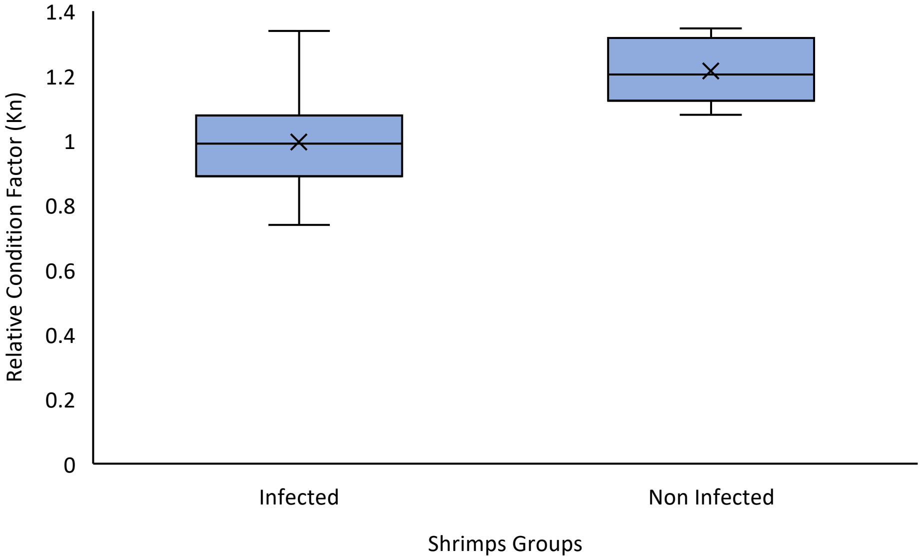
Appendix Figure S29: Carapace length- weight relationship of all non-Infected males *Palaemon paucidens* collected from Shimane prefecture during April 2018.



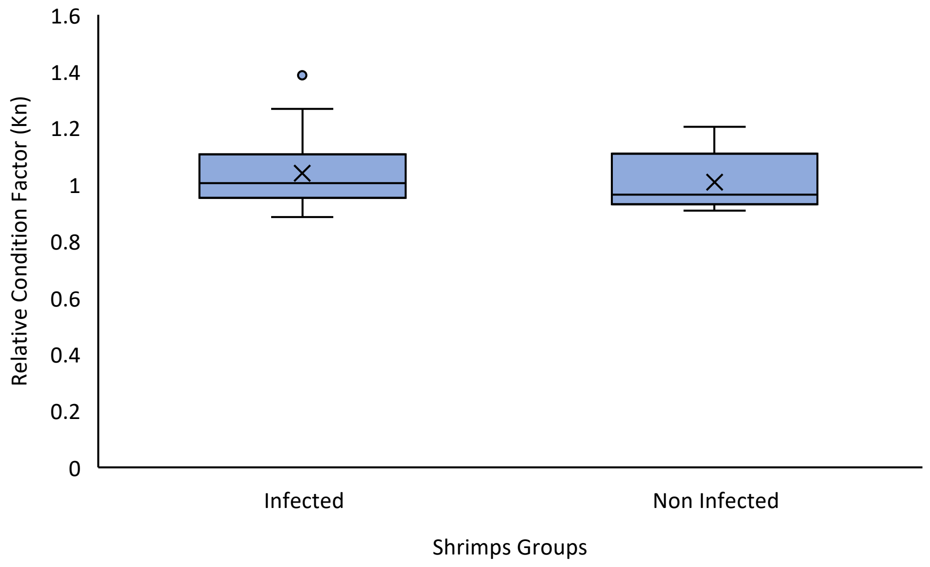
Appendix Figure S30: Carapace length- weight relationship of all Infected *Palaemon paucidens* collected from Shimane prefecture during April 2018.



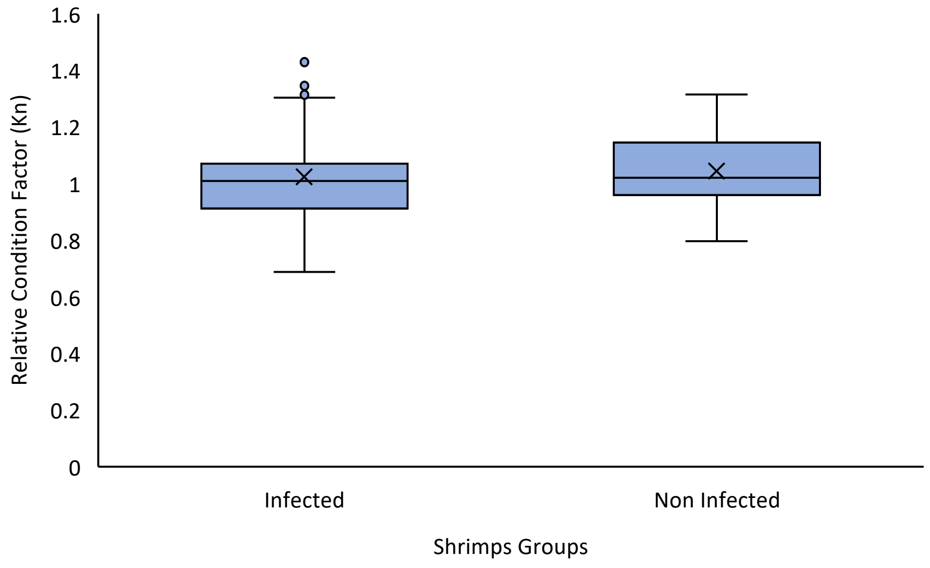
Appendix Figure S31: Carapace length- weight relationship of all non-Infected *Palaemon paucidens* collected from Shimane prefecture during April 2018.



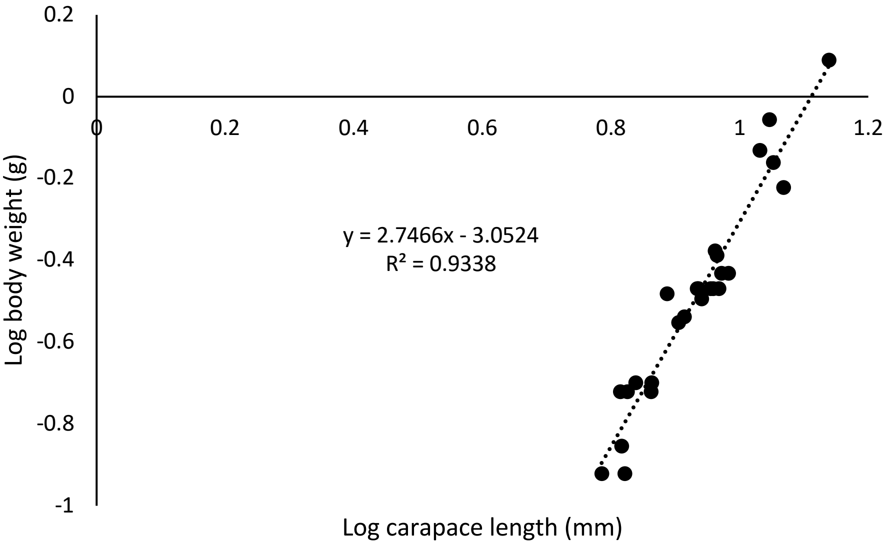
Appendix Figure S32: Boxplot of the relative condition factor (K_n) for infected and non-infected female *Palaemon paucidens* collected from Shimane prefecture during May 2018. Boxplots are combined with a mean, minimum, maximum, first quartile, median and third quartile values.



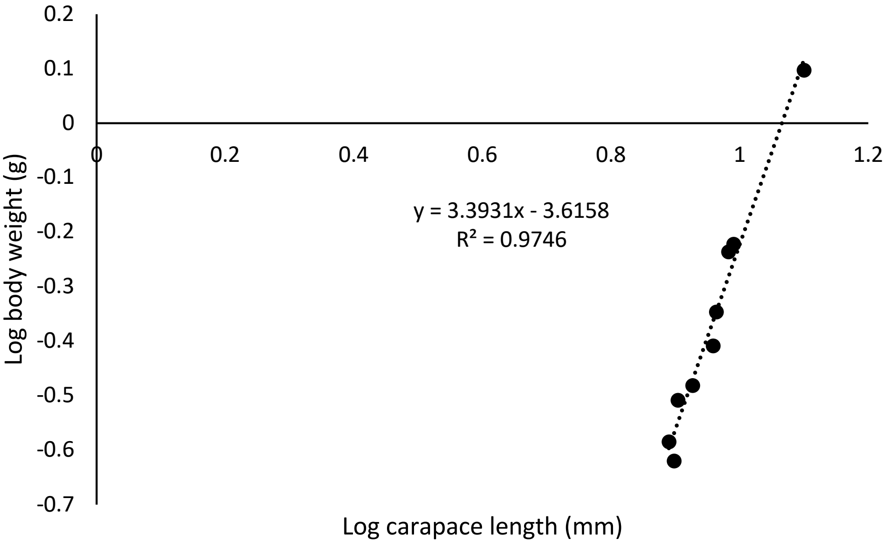
Appendix Figure S33: Boxplot of the relative condition factor (K_n) for infected and non-infected male *Palaemon paucidens* collected from Shimane prefecture during May 2018. Boxplots are combined with a mean, minimum, maximum, first quartile, median and third quartile values.



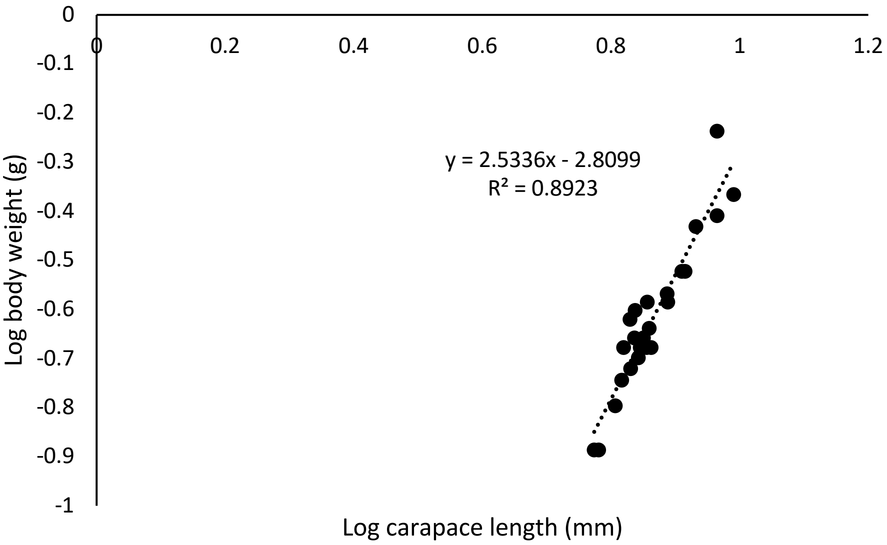
Appendix Figure S34: Boxplot of the relative condition factor (K_n) for infected and non-infected *Palaemon paucidens* collected from Shimane prefecture during May 2018. Boxplots are combined with a mean, minimum, maximum, first quartile, median and third quartile values.



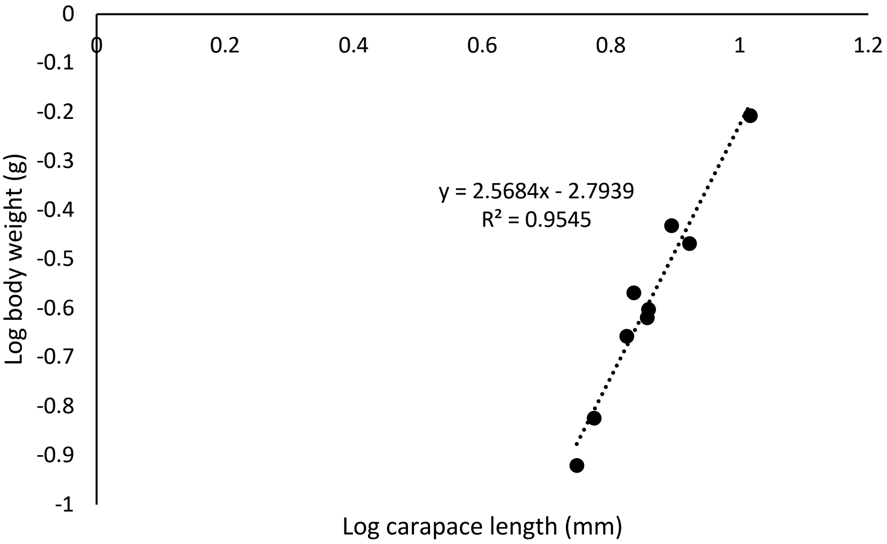
Appendix Figure S35: Carapace length- weight relationship of all Infected female *Palaemon paucidens* collected from Shimane prefecture during May 2018.



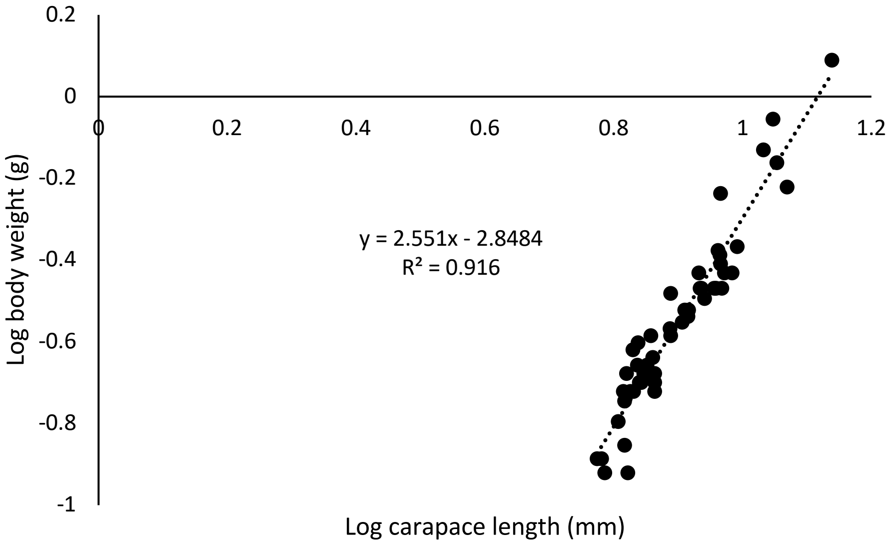
Appendix Figure S36: Carapace length- weight relationship of all non-Infected female *Palaemon paucidens* collected from Shimane prefecture during May 2018.



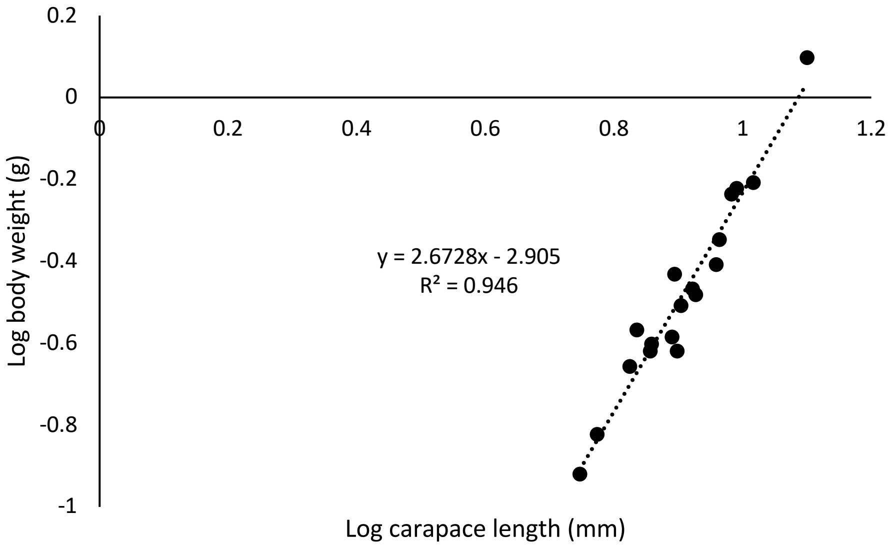
Appendix Figure S37: Carapace length- weight relationship of all Infected male *Palaemon paucidens* collected from Shimane prefecture during May 2018.



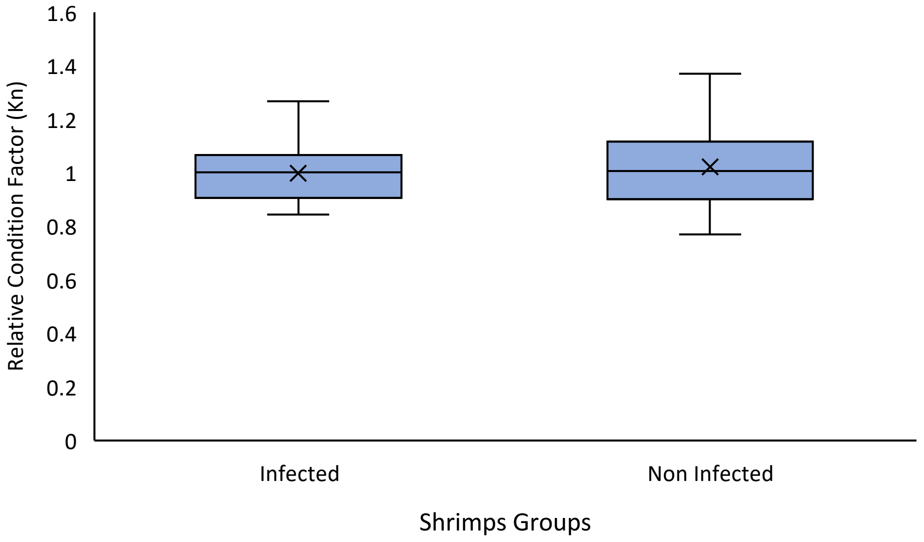
Appendix Figure S38: Carapace length- weight relationship of all non-Infected male *Palaemon paucidens* collected from Shimane prefecture during May 2018.



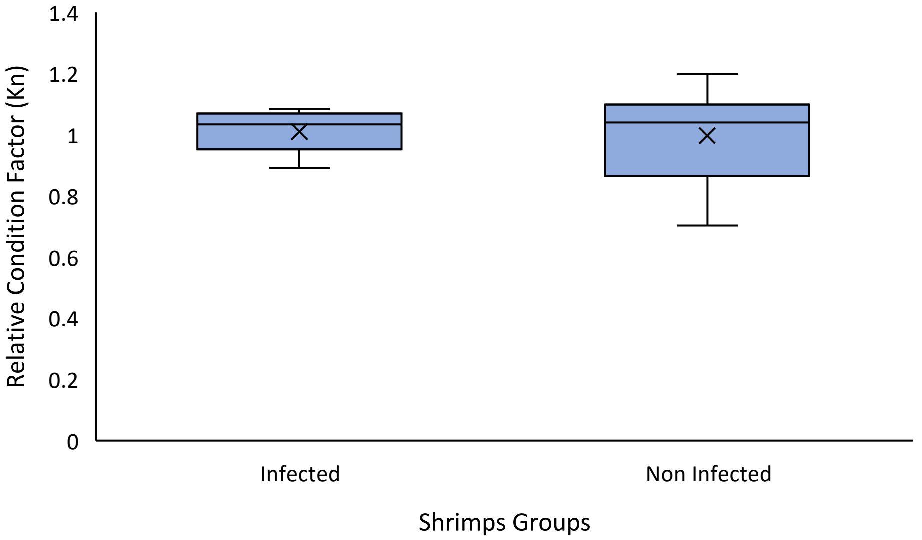
Appendix Figure S39: Carapace length- weight relationship of all Infected *Palaemon paucidens* collected from Shimane prefecture during May 2018.



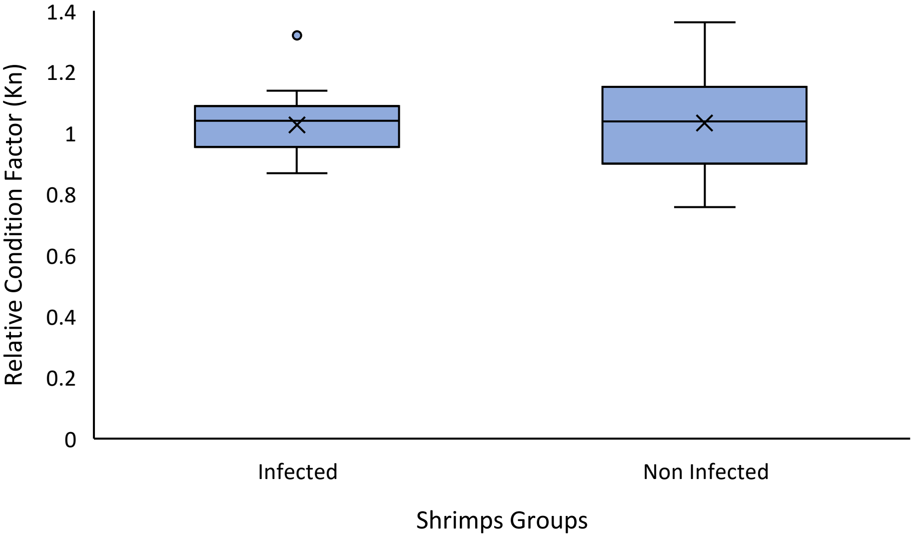
Appendix Figure 40: Carapace length- weight relationship of all non-Infected *Palaemon paucidens* collected from Shimane prefecture during May 2018.



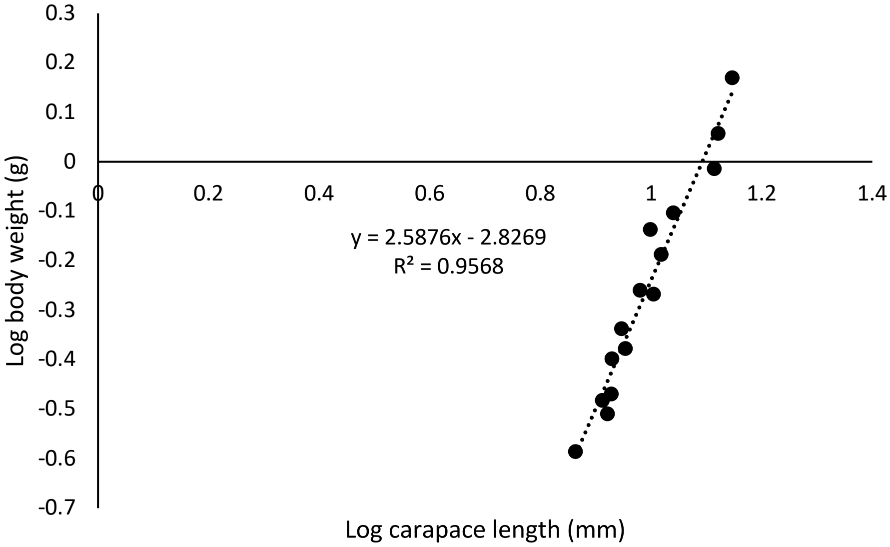
Appendix Figure S41: Boxplot of the relative condition factor (K_n) for infected and non-infected female *Palaemon paucidens* collected from Shimane prefecture during June 2018. Boxplots are combined with a mean, minimum, maximum, first quartile, median and third quartile values.



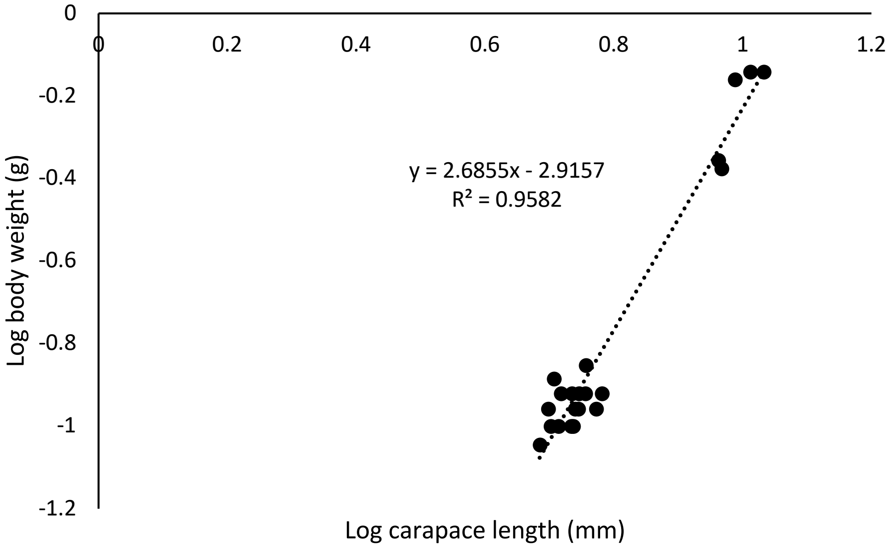
Appendix Figure S42: Boxplot of the relative condition factor (K_n) for infected and non-infected male *Palaemon paucidens* collected from Shimane prefecture during June 2018. Boxplots are combined with a mean, minimum, maximum, first quartile, median and third quartile values.



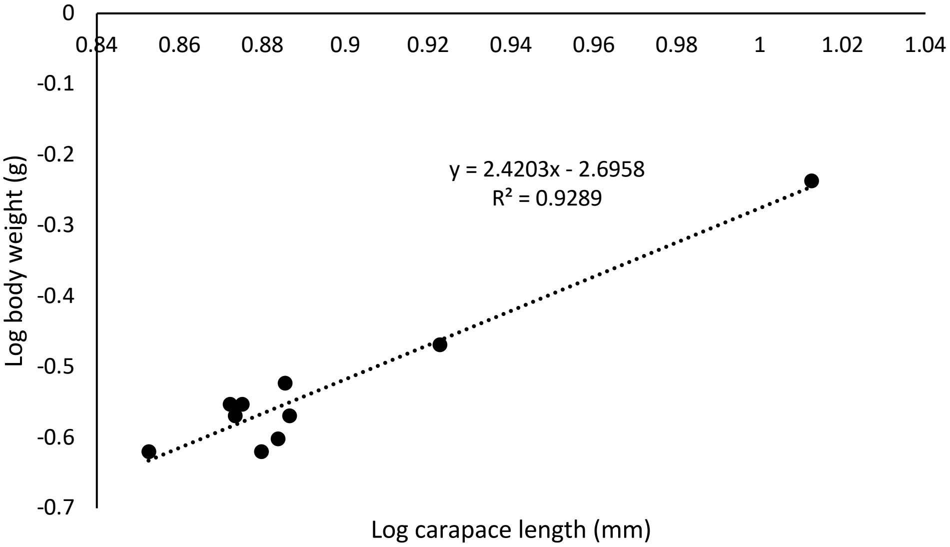
Appendix Figure S43: Boxplot of the relative condition factor (K_n) for infected and non-infected *Palaemon paucidens* collected from Shimane prefecture during June 2018. Boxplots are combined with a mean, minimum, maximum, first quartile, median and third quartile values.



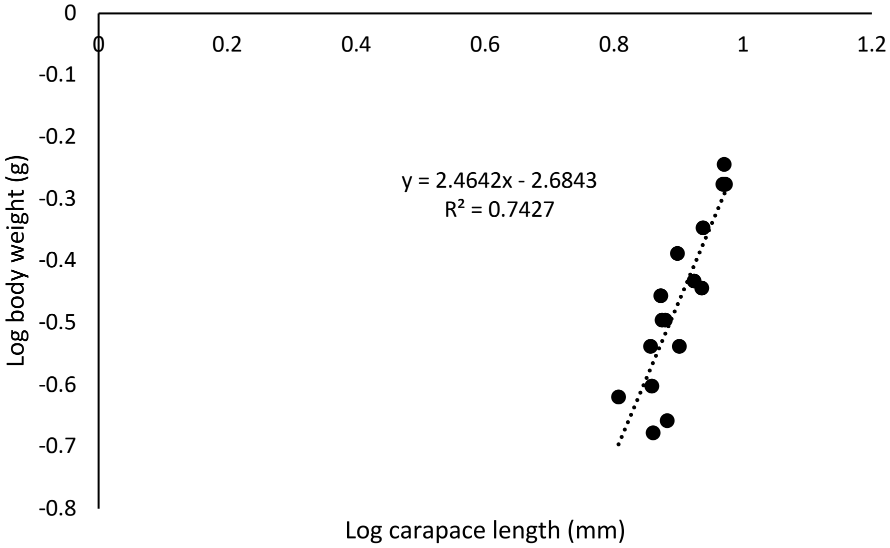
Appendix Figure S45: Carapace length- weight relationship of all Infected females *Palaemon paucidens* collected from Shimane prefecture during June 2018.



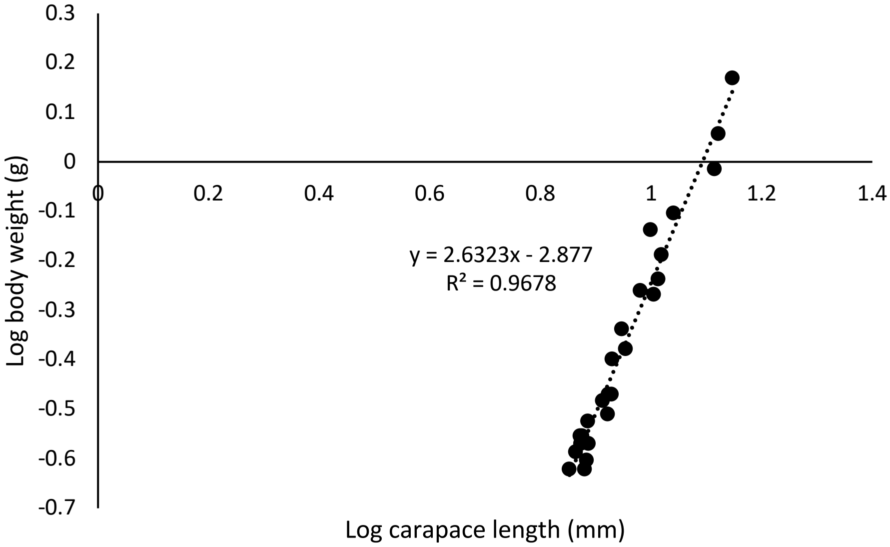
Appendix Figure S46: Carapace length- weight relationship of all non-Infected females *Palaemon paucidens* collected from Shimane prefecture during June 2018.



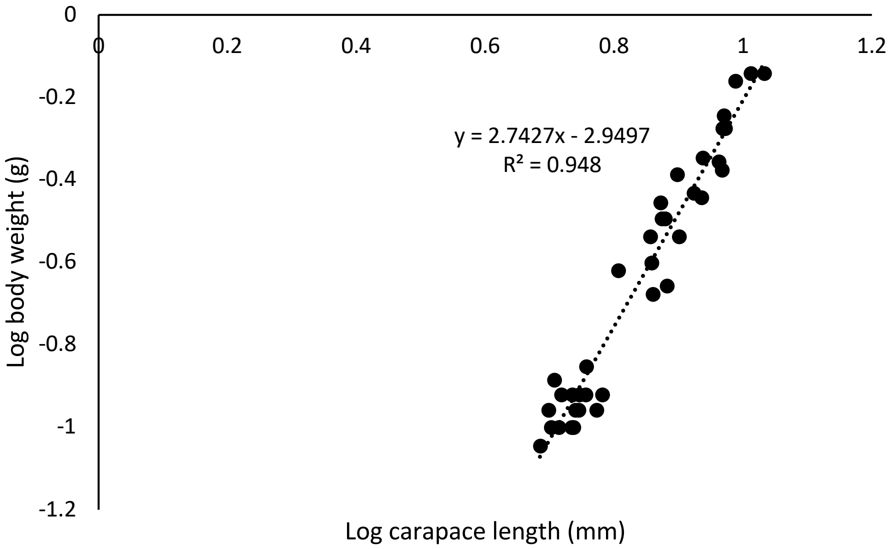
Appendix Figure S47: Carapace length- weight relationship of all Infected males *Palaemon paucidens* collected from Shimane prefecture during June 2018.



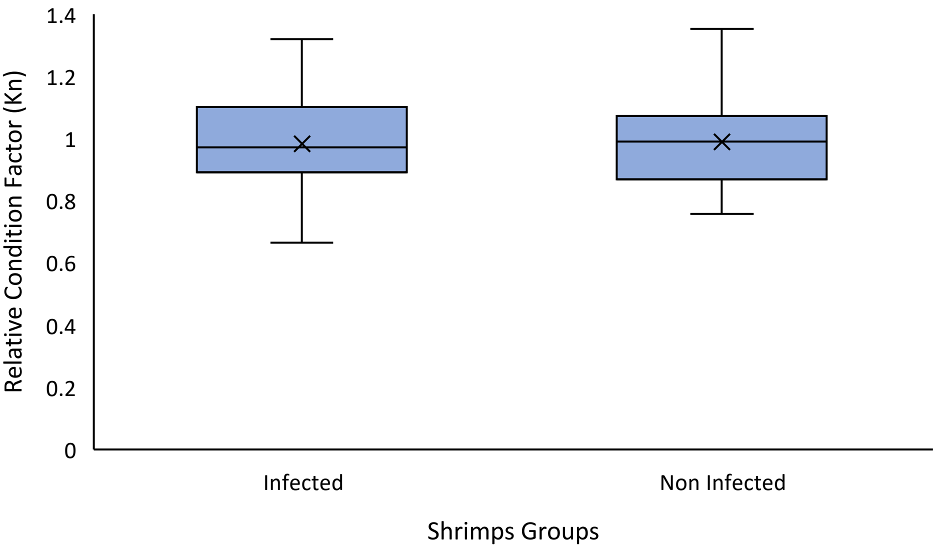
Appendix Figure S48: Carapace length- weight relationship of all non-Infected males *Palaemon paucidens* collected from Shimane prefecture during June 2018.



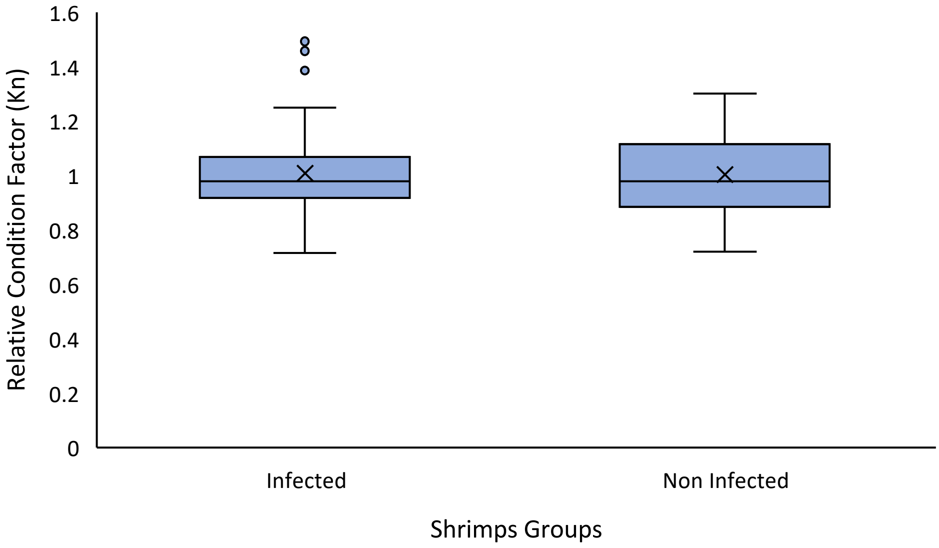
Appendix Figure S49: Carapace length- weight relationship of all Infected *Palaemon paucidens* collected from Shimane prefecture during June 2018.



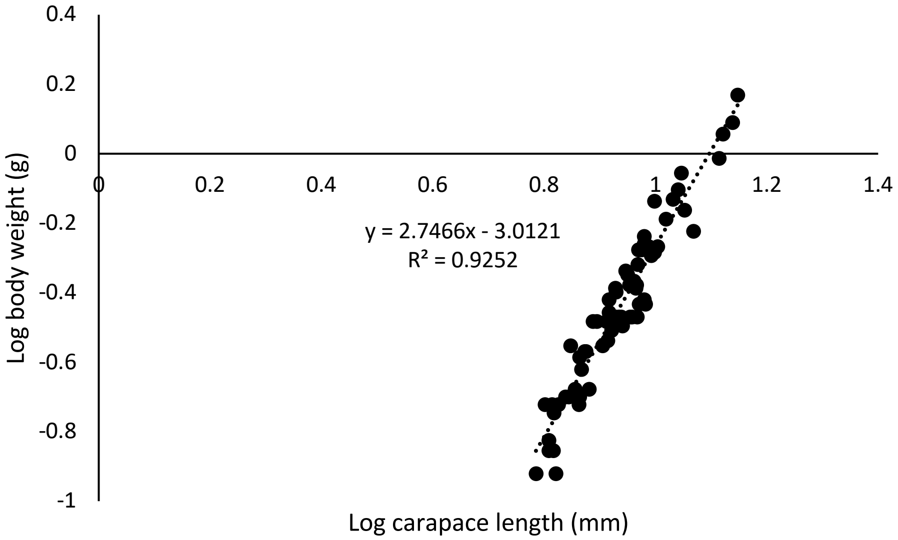
Appendix Figure S5: Carapace length- weight relationship of all non-Infected *Palaemon paucidens* collected from Shimane prefecture during June 2018.



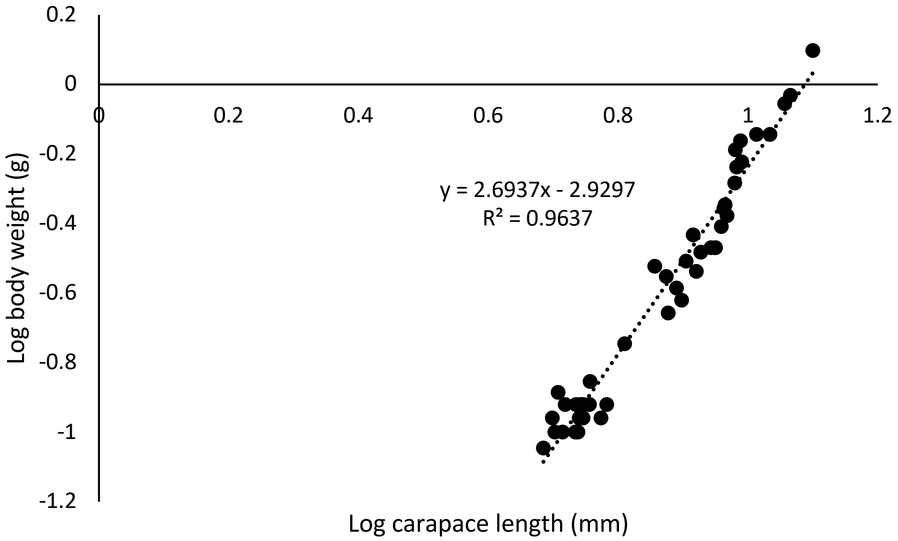
Appendix Figure S51: Boxplot of the relative condition factor (K_n) for infected and non-infected females *Palaemon paucidens* collected from Shimane prefecture during April to June 2018. Boxplots are combined with a mean, minimum, maximum, first quartile, median and third quartile values.



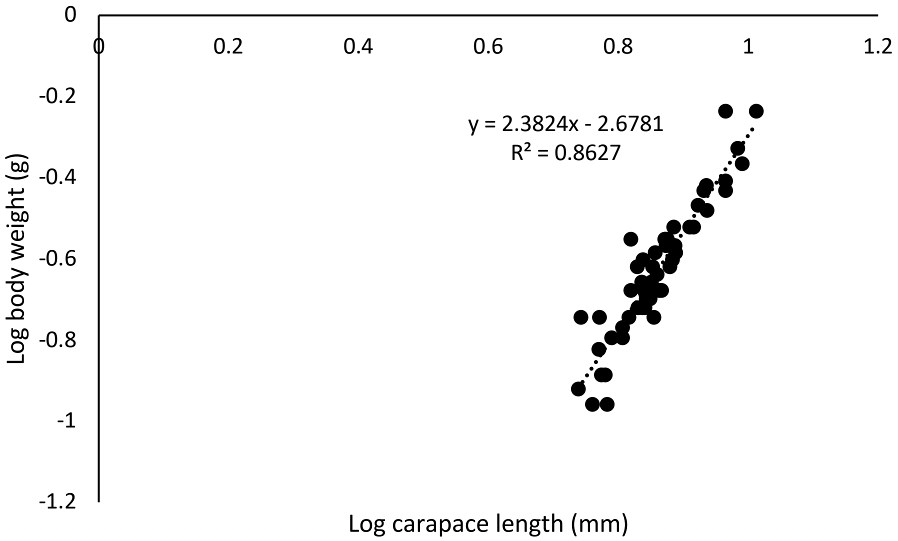
Appendix Figure S52: Boxplot of the relative condition factor (K_n) for infected and non-infected males *Palaemon paucidens* collected from Shimane prefecture during April to June 2018. Boxplots are combined with a mean, minimum, maximum, first quartile, median and third quartile values.



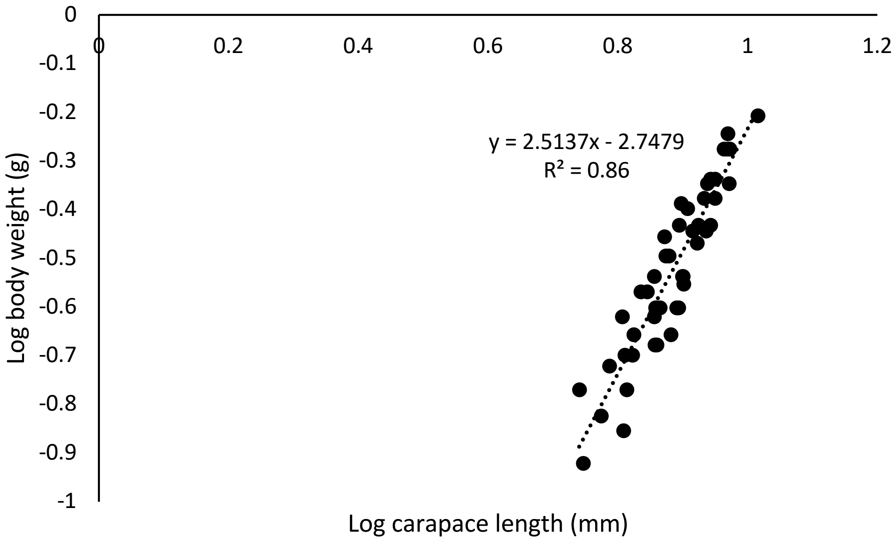
Appendix Figure S53: Carapace length- weight relationship of all Infected female *Palaemon paucidens* collected from Shimane prefecture during April to June 2018.



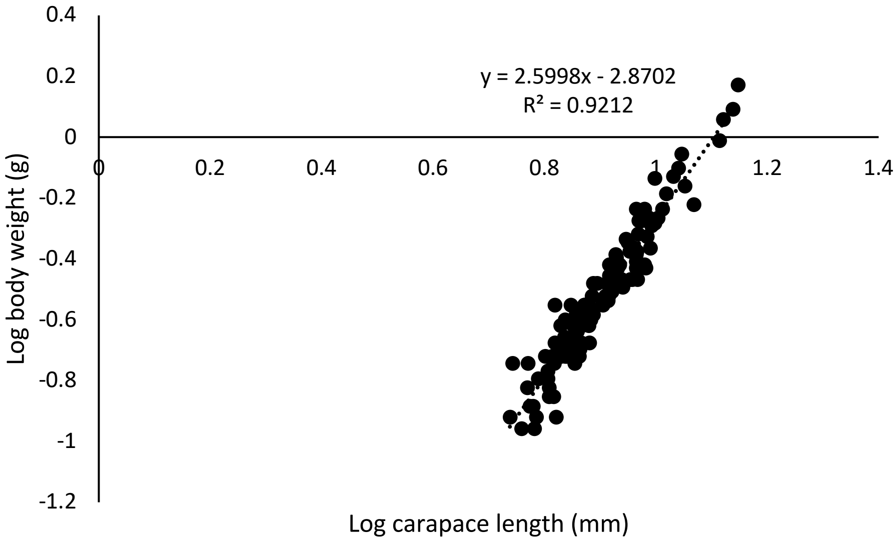
Appendix Figure S54: Carapace length- weight relationship of all non-Infected female *Palaemon paucidens* collected from Shimane prefecture during April to June 2018.



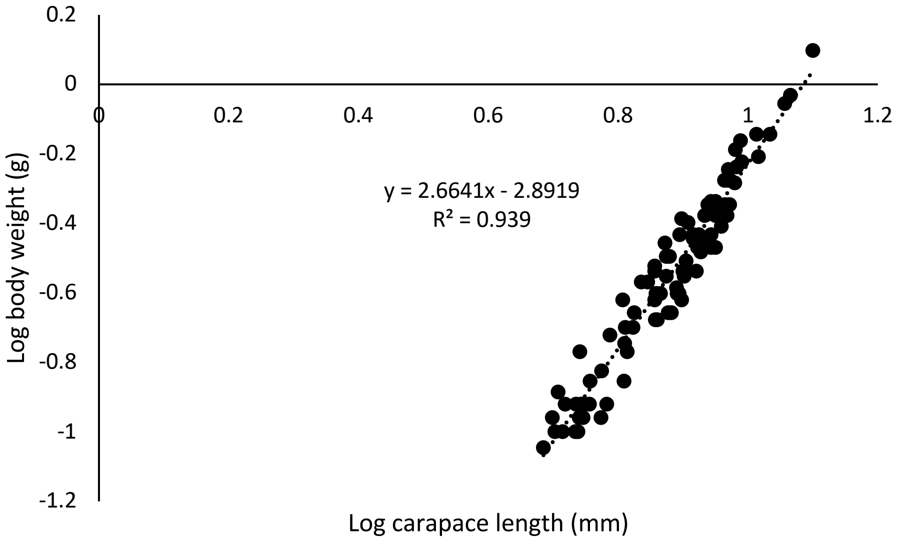
Appendix Figure S55: Carapace length- weight relationship of all Infected males *Palaemon paucidens* collected from Shimane prefecture during April to June 2018.



Appendix Figure S56: Carapace length- weight relationship of all non-Infected males *Palaemon paucidens* collected from Shimane prefecture during April to June 2018.



Appendix Figure S57: Carapace length- weight relationship of all Infected *Palaemon paucidens* collected from Shimane prefecture during April to June 2018.



Appendix Figure S58: Carapace length- weight relationship of all non-Infected *Palaemon paucidens* collected from Shimane prefecture during April to June 2018.