Zoological Studies **64:**64 (2025)

Multiple Infestation of Ectoparasitic Isopods *Tachaea chinensis* on the Freshwater Shrimp *Palaemon paucidens* in Shimane Prefecture, Japan

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(Received 11 July 2025 / Accepted 22 October 2025 / Published -- 2025)

Communicated by Hsi-Te Shin

Tachaea chinensis Thielemann, 1910 is a species of ectoparasitic isopod that infests freshwater shrimps and prawns. Individuals are often found attached to the side of their host shrimp's carapace. Despite the limited space for attachment there, records indicate that several isopods can infest a single host shrimp simultaneously. There are limited data, however, on the prevalence of such occurrences and their potential effect on host survival. Here, we report on the occurrence of multiple infestation by *T. chinensis* on the freshwater shrimp *Palaemon paucidens* De Haan, 1849 in

Izumo, Shimane Prefecture, Japan, during April and May of 2018 and 2019. The prevalence of infested shrimp was higher (80.0–90.2%) at St. 1, a semi-closed spillway that alternates between continuous water flow and isolated stagnancy, than at St. 2 (45.0–55.0%), an open stream with year-round continuous water flow. The number of parasites per host was zero to nine at St.1 and zero to three at St. 2. A significant positive correlation was observed between the prevalence of isopods on shrimp and the mean number of these parasites per host. In a laboratory experiment in which individual shrimp of three size groups were reared for seven days with different numbers of isopods from three matching size groups, the survival rate of hosts was 100% with one *T. chinensis*, 90–100% with two, 50–80% with four, and 10–40% with eight. These results suggest that the presence of two or more parasites negatively affects the hardiness of the host, increasing shrimp mortality and potentially reducing their density in nature, especially in semi-closed habitats. This has obvious implications for shrimp farms, which should strive to prevent the entry of this isopod into their water systems.

Keywords: Freshwater shrimp, Parasitic isopod, Host-parasite relations, Parasite prevalence, Host survival rate

Citation: Suenaga A, Khalfan AWM, Yakushijin D, Ogasawara C, Tani S, Imai T, Saito H. 2025. Multiple infestation of ectoparasitic isopods *Tachaea chinensis* on the freshwater shrimp *Palaemon paucidens* in Shimane Prefecture, Japan. Zool Stud **64:**64.

BACKGROUND

Tachaea Schioedte & Meinert, 1879 is a small genus of corallanid isopods, with nine described species around the world including one that occurs in Japan (Nunomura and Shimomura 2020; also cite WoRMS). *Tachaea chinensis* Thielemann, 1910 has been reported from Japan, China, Vietnam, Thailand, and Malaysia. It is a hemolymph-feeding ectoparasite on the carapace of freshwater shrimps and prawns belonging to the atyid genera *Caridina* H. Milne Edwards, 1837, *Neocaridina*

Kubo, 1938, and *Paratya* Miers, 1882, the palaemonid genera *Macrobrachium* Spence Bate, 1868 and *Palaemon* Weber, 1795, and the penaeid genus *Penaeus* Febricius, 1798 (Nagasawa et al. 2018; Xu et al. 2019 2021; Nunomura and Shimomura 2020; Li et al. 2021; Khalfan et al. 2022 2023a 2023b; Maeda and Urabe 2022). Elucidating the parasitic ecology of these isopods is important for understanding the interactions between hosts and parasites in the ecosystem (Ota 2023). *T. Chnensis* showed the biphasic lifestyle: isopods in the manca stage and the subsequent immature stage infest shrimp whereas mature isopods detach and engage in reproductive activity (Ota 2019). Unlike cymothoid isopods, *T. chinensis* does not tightly grasp the body of its host, but quickly detaches when stimulated and actively swims away (Takeda et al. 2000; Ota 2023). Xu et al. (2021) furthermore observed that upon that the death of a host shrimp, *T. chinensis* invariably detaches from it and begins searching for a new host.

Host body size and position on the host are important factors in host selection. Khalfan et al. (2023a) found that *T. chinensis* preferred to attach to shrimp with a carapace equal in length to the isopod or up to 43% longer; they suggested that this is enough space for safe attachment while avoiding predation by other shrimp. Infested shrimp usually have 1, or rarely 2 or 3 isopods when enough space is available (Nagasawa et al. 2018; Ota 2019; Li et al. 2020b; Khalfan et al. 2022). Up until now, there have been no reports of four or more isopods attached to a single host shrimp in the natural environment.

We have found numerous isopods, up to nine individuals, on single specimens of the shrimp *Palaemon paucidens* De Haan, 1844 in Shimane Prefecture, Japan. Here, we compare the prevalence of infestation and the number of parasitic isopods per host, i.e. the intensity of infestation, at two sites. Moreover, we investigated the effect of the number of attached *T. chinensis* on the survival rate of their hosts in the laboratory to establish whether these parasites may be regulating the host population in the field.

MATERIALS AND METHODS

Field Survey

The survey was conducted at two freshwater sampling sites in Izumo City, Shimane Prefecture, on 18 April and 11 May 2018 and on 25 April and 24 May 2019. In spring, the isopods are fully grown and it is easy to count them on individual host shrimp (Fig. 1A). Station 1 (St. 1; 35°26'57"N, 132°47'51"E) is a spillway (10 m long × 2 m wide × ca 0.5 m deep) for drawing excess water from an irrigation pond down to a tributary stream of the Mizutani River in the Hii River system (Fig. 1B). The spillway is situated 3 m below the pond and 0.5 m above the stream. Depending on the pond's water level, its spillway may experience continuous flow or contain an isolated, stagnant water body, and may therefore be termed semi-closed (Fig. 1D, E). At the time of collection, the spillway was cut off from the pond on 2018, while it was running freely on 2019. Preliminary surveys showed that the host shrimp *P. paucidens* occurs with the parasite *T. chinensis* in both the irrigation pond and the spillway, but the latter was selected as this study's collection site (St. 1) because the shrimp and isopods could be collected more quickly there. Station 2 (St. 2; 35°27'02"N, 132°47'53"E) is situated farther downstream along the same outflow stream (stream width and depth ca. 1.5 m and 0.5 m respectively). This an open environment with continuous water flow year-round (Fig. 1C). At both sites, specimens of *P. paucidens* and its parasite *T. chinensis* were scooped up in a hand net (mouth diameter 30 cm, mesh size 2.5 mm, handle length 5 m) from an area of ca. 5 m^2 along a concrete bank. All host shrimp were placed in individual bags with their parasites and immediately preserved in 70% ethanol.

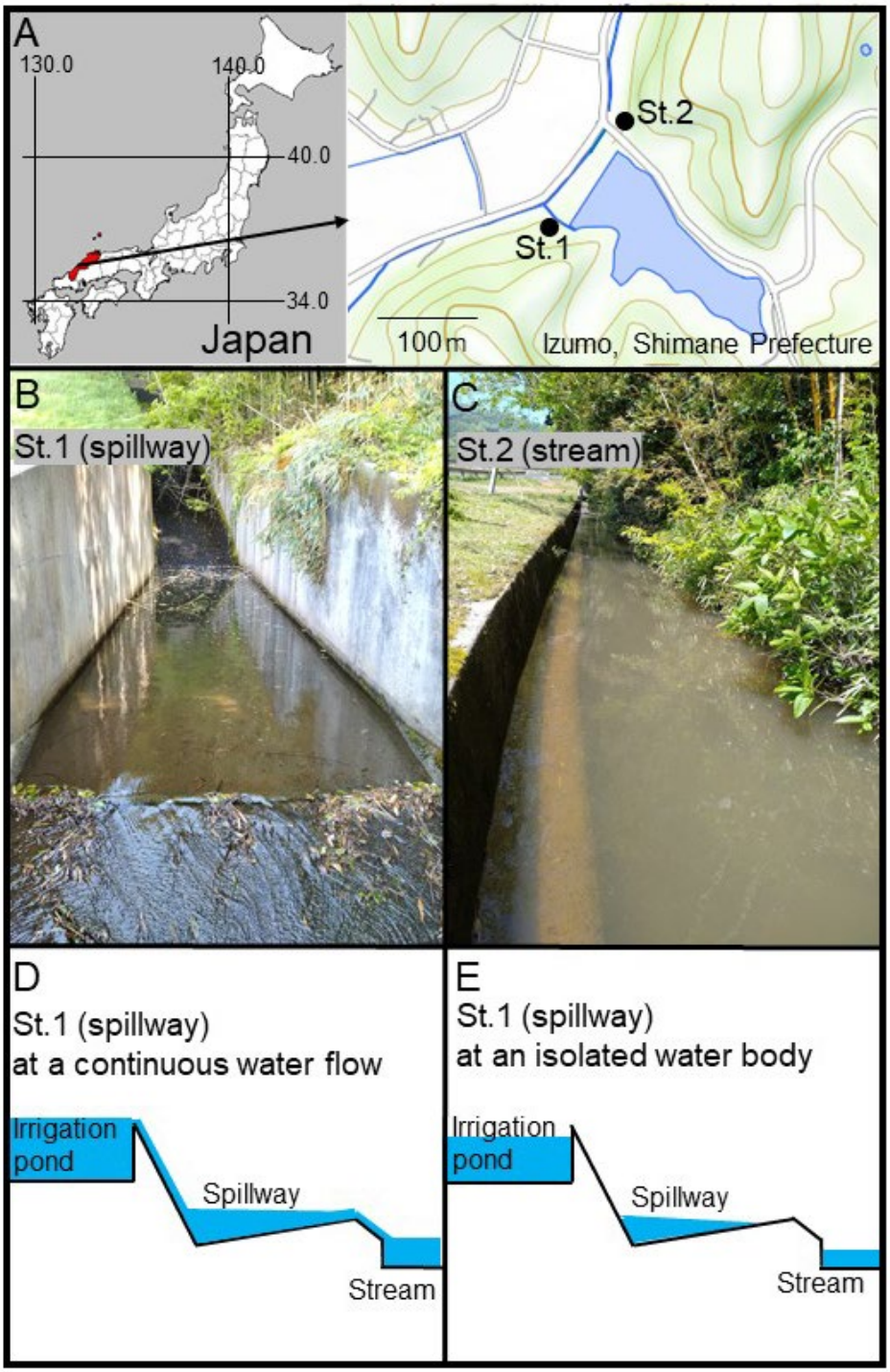

Fig. 1. Sampling sites of the ectoparasitic isopod *Tachaea chinensis* and host shrimp *Palaemon paucidens* in Izumo, Shimane Prefecture, Japan. (A) Map of the site. (B) Photograph of St. 1 (spillway). (C) Photograph of St. 2 (stream). (D) Schematic diagram of St. 1 (spillway) in continuous flow. (E) Schematic diagram of St. 1 (spillway) as an isolated water body.

In the laboratory, the shrimp were divided into males (those with fully developed penes) and females (those with a brood pouch). The carapace length of each shrimp was measured to the nearest 0.1 cm, using a digital caliper, from the orbital margin to the posterior margin. Presence or

absence of *T. chinensis* was recorded for each shrimp, and if *T. chinensis* was present, the number of isopods was counted and the total length of each was measured, again using a digital caliper, from the tip of the head to the end of the uropods. Prevalence, expressed as a percentage, was calculated as the number of shrimp bearing at least one ectoparasite divided by the total number of shrimp.

Laboratory experiment

Specimens of *P. paucidens* with or without *T. chinensis* attached were collected using a hand net from June 2023 to September 2025 (except ovigerous female shrimp) at the two freshwater sites described above (St. 1 and St. 2 in Izumo City, Shimane Prefecture). The collected shrimp and parasites were placed in water-filled containers fitted with aeration equipment and transported to the laboratory. There, infested and uninfested shrimp were kept in two large tanks (size) of filtered water separately, where they were fed commercial shrimp food (Hikari Ranchu Disc, 1.3–1.5 mm, Kyorin, Himeji, Hyogo, Japan) for no more than a week before experimental use.

Only uninfested shrimp were used for experiments. Isopods were used that had been carefully peeled from their host shrimp and placed in 500 ml plastic containers with aeration. Bearing in mind the isopods' preferred host-parasite size ratio (1:1 to 10:7; see Khalfan et al. 2023a), both shrimp and isopods were divided into three size categories as follows: large (hosts 9–9.5 mm in carapace length, parasites 7–7.5 mm in body length), medium (hosts 7–7.5 mm, parasites 5.5–6.0 mm), and small (hosts 5.0–5.5 mm, parasites 4.0–4.5 mm). Within each size group, a rearing experiment was conducted with zero, one, two, four, or eight isopods paired with each host shrimp to investigate the effect of the number of parasites on the survival of the host. The isopods were starved for 24h prior to experimentation. In each trial, the shrimp and parasite(s) were simultaneously placed in a plastic tank (13 cm × 20 cm × 13 cm) filled with 2 L of water with aeration. Different specimens of both species were used in each trial to avoid the effects of repeated exposure in experiments. Each trial was conducted in the dark, without food, at 24 ± 1°C. The position(s) of the isopod(s) on the host shrimp's body and the condition of the shrimp (alive or dead) were monitored once a day for up to seven days. Ten replicates of each trial set-up were conducted, therefore 150 trials in all.

Statistical analysis

Variation in the numbers of parasites per host at the two sampling stations in the field study was tested by the Mann-Whitney U-test. Variation in the prevalence at the two sampling stations in the field study was tested by the Fisher exact text. The relationship between the prevalence and the mean number of parasites per host was calculated by the Spearman rank correlation coefficient. The survival time of host shrimp with different numbers of parasites in the laboratory experiment was tested using the Kruskal-Wallis test coupled with a Steel-Dwass test. All statistical processing was performed using the statistical software EZR version 1.68 (Kanda 2013).

RESULTS

Field survey

In 2018, 41 individuals of the host shrimp *Palaemon paucidens* were collected at St. 1 (19 in April, 22 in May), and 20 at St. 2 (11 in April, 9 in May); in 2019, 50 shrimp were collected at St. 1 (34 in April, 16 in May), and 60 at St. 2 (38 in April, 22 in May). In 2018, the overall prevalence of the ectoparasitic isopod *Tachaea chinensis* on these shrimp (Table 1) was 90.2% at St. 1 (89.5% in April, 90.9% in May) and 45.0% at St. 2 (54.5% in April, 33.3% in May); in 2019, the overall prevalence was 80.0% at St. 1 (88.2% in April, 62.5% in May) and 55.0% at St. 2 (52.6% in April, 59.1% in May). The isopod was thus more prevalent at St. 1 than at St. 2 during the spring in both years (Fisher exact text, $P = 0.00092$ in 2018, $P = 0.0081$ in 2019). In 2018, the number of parasitic isopods per shrimp was zero to nine individuals at St. 1, and zero to three individuals at St. 2, in 2019, the comparable numbers were zero to four individuals at St. 1 and zero to two individuals at St. 2 (Fig. 2). In both years the number of isopods per shrimp was significantly higher at St. 1 than at St. 2 (Mann-Whitney U test, $P = 0.000011$ in 2018, $P = 0.00071$ in 2019).

Table 1. Numbers and prevalence of the ectoparasitic isopod *Tachaea chinensis* on the host shrimp *Palaemon paucidens* in Shimane Prefecture, Japan

	Host shrimp *Palaemon paucidens*							Ectoparasite *Tachaea chinensis*		
Station	Date	Number of males	Number of females	Mean carapace length of males (mm)	Mean carapace length of females (mm)	Number infested	Prevalence (%)	Mean infestation (ind)	Maximum infestation (ind)	Mean body length (mm)
St. 1	18 April 2018	11	8	6.22	7.33	17	89.5	3.47	9	6.05
	11 May 2018	12	10	6.99	7.29	20	90.9	2.18	5	6.55
	Mean	11.5	9	6.62	7.31	18.5	90.2	2.78	7	6.26
St. 2	18 April 2018	8	3	7.51	8.56	6	54.5	0.82	2	6.40
	11 May 2018	3	6	7.27	9.40	3	33.3	0.56	3	8.86
	Mean	5.5	4.5	7.45	9.12	4.5	45.0	0.70	2.5	7.28
St. 1	25 April 2019	29	5	6.53	8.21	30	88.2	1.26	4	6.48
	24 May 2019	12	4	7.09	9.34	10	62.5	0.81	3	7.77
	Mean	20.5	4.5	6.78	8.71	20	80.0	1.12	3.5	6.78
St. 2	25 April 2019	23	15	6.96	9.14	20	52.6	0.58	2	6.57
	24 May 2019	9	13	7.34	9.83	13	59.1	0.68	2	7.52
	Mean	16.0	14.0	7.07	9.46	16.5	55.0	0.62	2	6.95

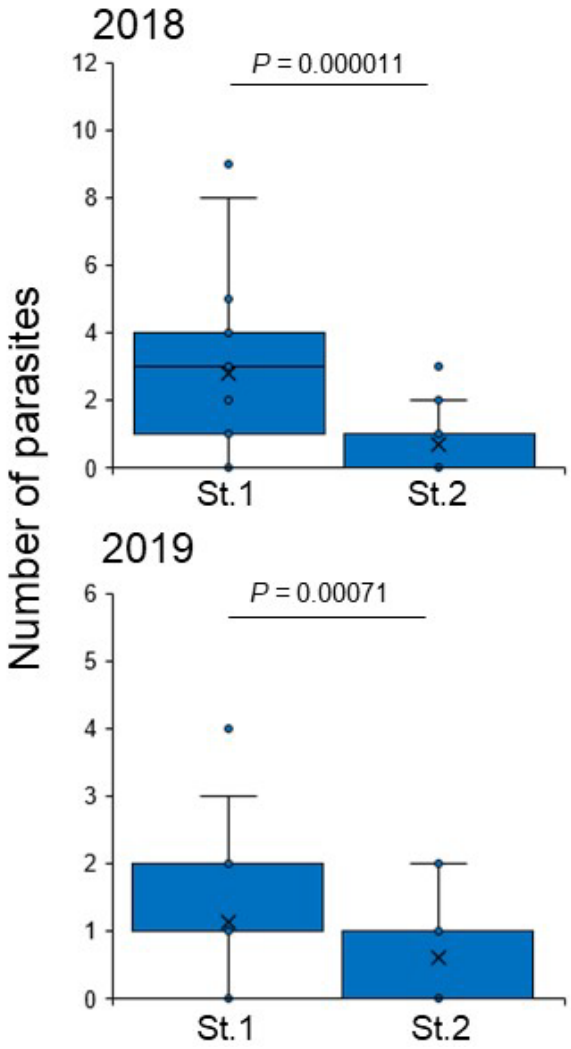

Fig. 2. Comparison of numbers of ectoparasitic isopod *Tachaea chinensis* per host shrimp *Palaemon paucidens* at Sts. 1 and 2 in 2018 and 2019. Data for April and May of each year are combined.

The most heavily infested specimen of *P. paucidens*, a female of CL 9.6 mm collected at St. 1 on 18 April 2018 (Fig. 3), bore nine *T. chinensis*, five on its left side, four on the right, which were attached to both the host's carapace and its abdomen. The shrimp appeared sluggish, and tissue damage was clearly visible on both sides along its entire body (Fig. 4A, B). In contrast, on a smaller male shrimp (CL 5.8 mm) that bore just one isopod, parasitic wounds were limited to one side of the carapace (Fig. 4C, D).

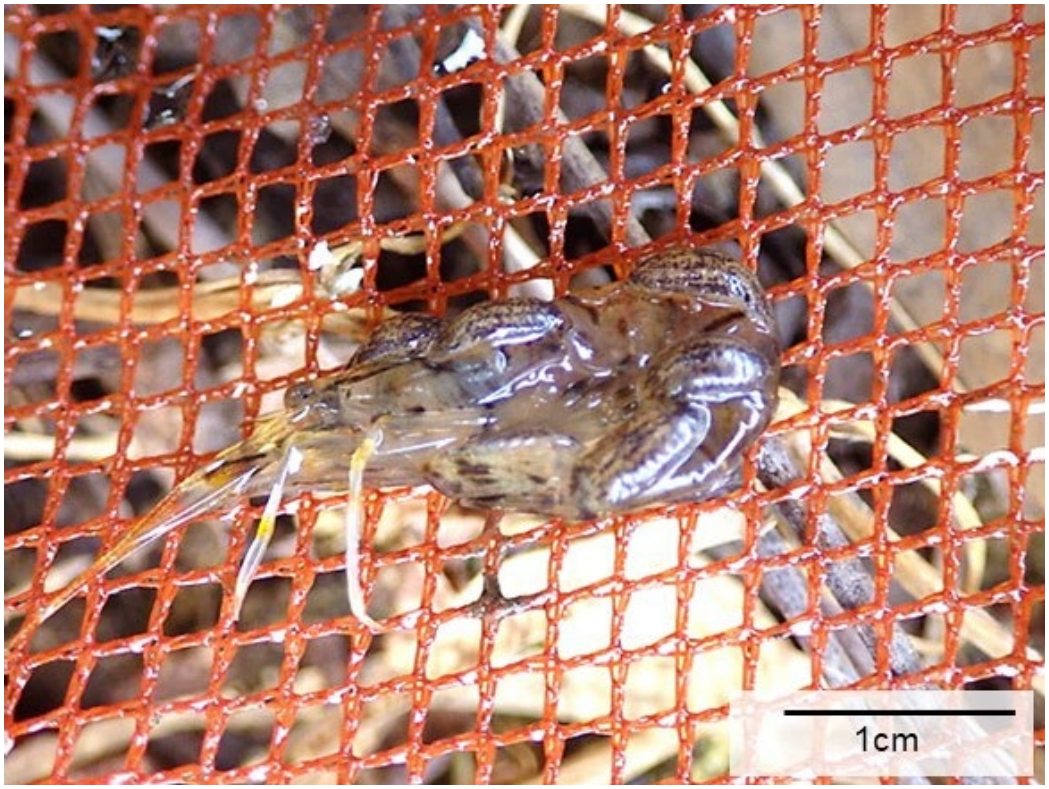

Fig. 3. Mass infestation of the parasite *Tachaea chinensis* on a specimen of the freshwater shrimp *Palaemon paucidens*. Five isopods are attached to the near side of the shrimp, and four more to its far side.

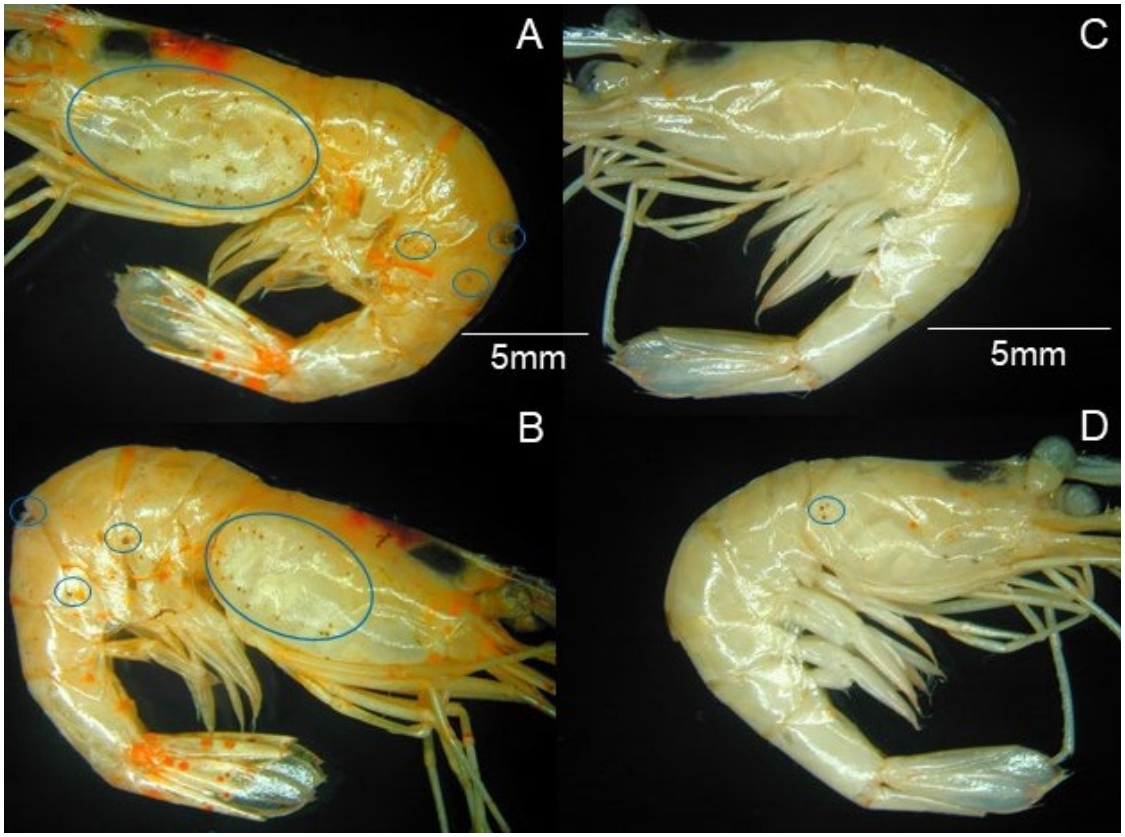

Fig. 4. Distribution of tissue damage (spots within ellipses) in *Palaemon paucidens* infected by *Tachaea chinensis*. (A, B) Both sides of a shrimp bearing nine parasites. (C, D) Both sides of a shrimp bearing one parasite.

There was a significant positive correlation between the prevalence during the survey period and the mean number of parasites per host (Spearman rank correlation: rho = 0.905, $P = 0.0046$). The mean number of parasites was approximately one individual at 60% prevalence, but it exceeded two individuals when prevalence increased to 85% (Fig. 5).

At St. 2, there were no occurrences of four or more parasites attached to one host shrimp at in either 2018 or 2019; in contrast, at St. 1, one shrimp (2%) was so infested in 2019 and a remarkable twelve individuals (29.3%) suffered such multiple infestations in 2018 (Fig. 6). That year, a significant positive correlation was found at St. 1 between shrimp carapace length and the number of parasites per host (including zero) ($P = 0.000032$; Fig. 6).

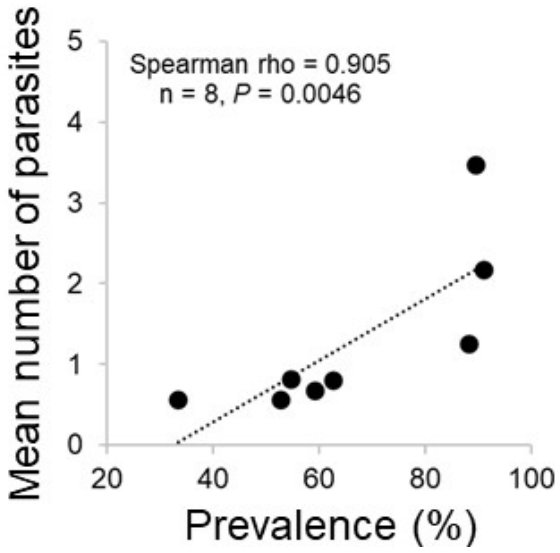

Fig. 5. Relationship between prevalence and mean numbers of parasitic isopods, *Tachaea chinensis*, per host shrimp, *Palaemon paucidens* at both Sts. 1 and 2 in 2018 and 2019, with the data for April and May each year shown separately.

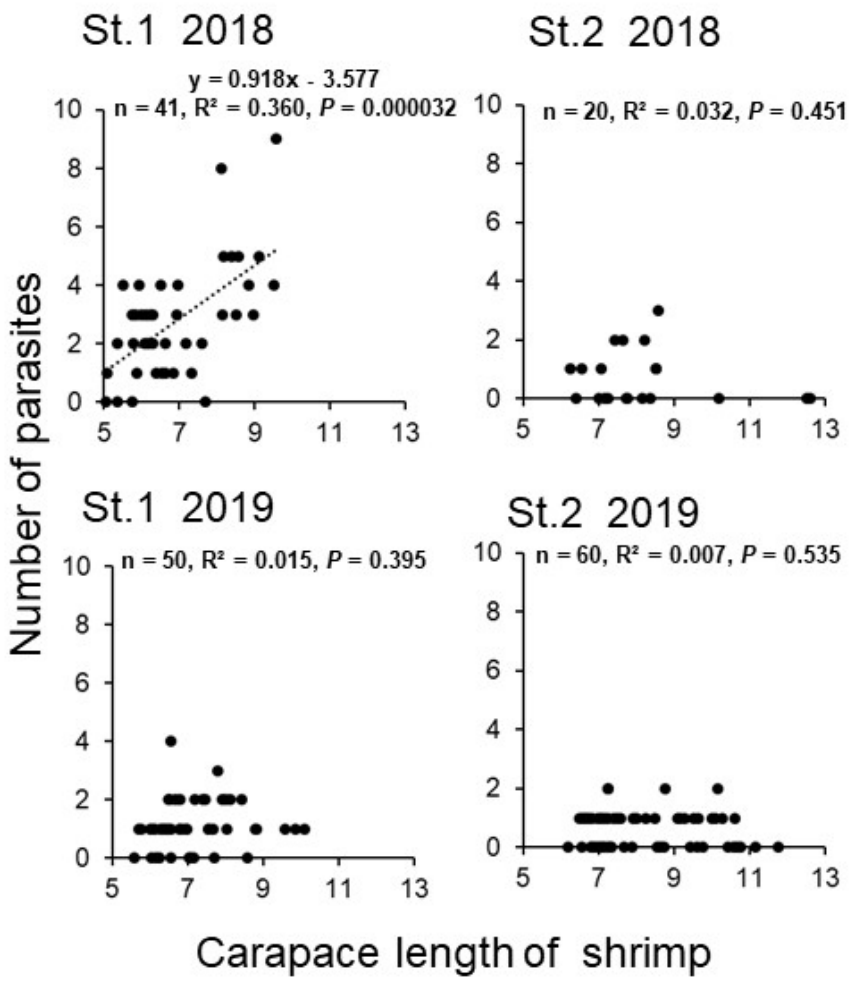

Fig. 6. Relationship between carapace length of the host shrimp and the number of parasitic isopods per host at Sts. 1 and 2 in 2018 and 2019. Data for April and May in each year are combined.

Laboratory experiment

Figure 7 compares the sites of infestation on individual host shrimp *Palaemon paucidens* exposed to different numbers of the parasitic isopod *Tachaea chinensis*. In trials with one or two isopods, they mainly attached to the shrimp's carapace regardless of size (large size group: 92.9–100%; medium size group: 82.9–95.7%; small size group: 97.1–97.8%). In trials with four or eight isopods, usually fewer than half were attached to the shrimp's carapace (large size group: 37.5–53.7%; medium size group: 29.0–64.3%; small size group: 27.2–63.0%) while others were found mostly attached to the abdomen (large size group: 4.3–39.0%; medium size group: 3.9–34.0%; small size group: 0.7–30.0%), but also to the rostrum/eyestalk, the tail fan, or, especially in eight-isopod trials, unattached.

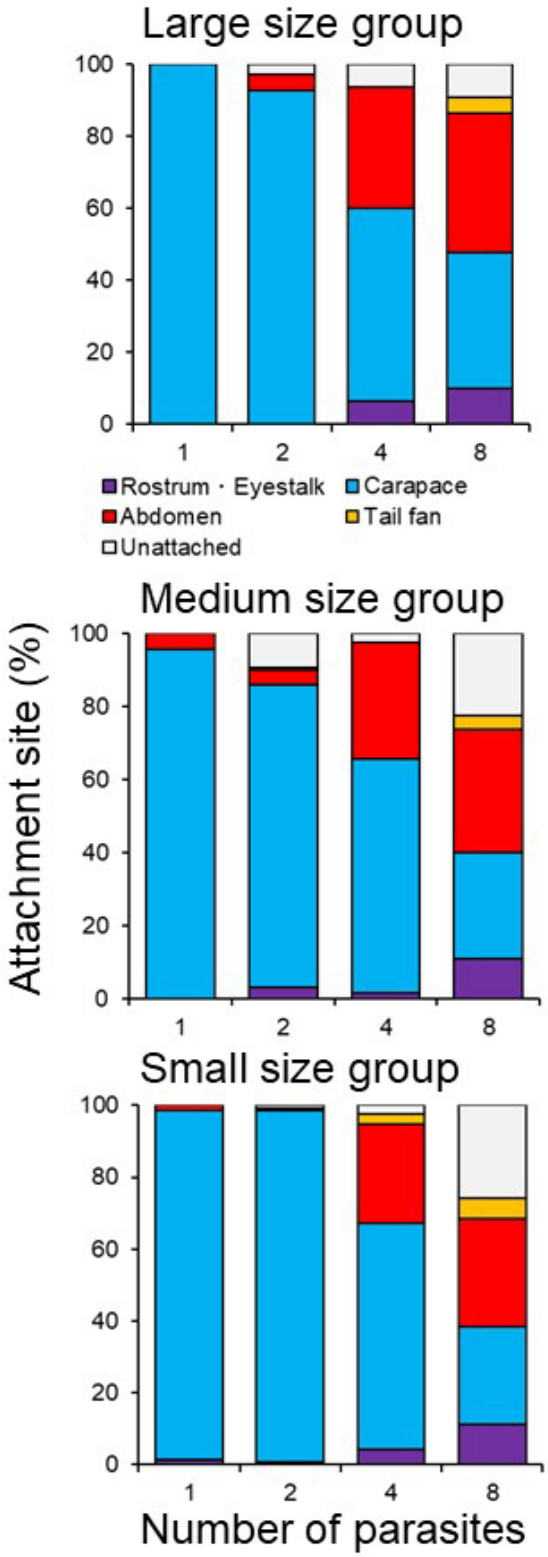

Fig. 7. Distribution of attachment sites of the ectoparasitic isopod *Tachaea chinensis* on three size-classes of the host shrimp *Palaemon paucidens* experimentally exposed to different numbers of correspondingly sized parasites.

Figure 8 compares the survival rate on day 7 and the mean survival times (with a seven-day cut-off) of host shrimp *P. paucidens* exposed to different numbers of the ectoparasite *T. chinensis*. In the

large size group, these values were 100% and 7.0 days for trials with zero, one or two isopods, 80% and 6.1 days for trials with four isopods, and 40% and 4.5 days for trials with eight isopods. The differences in mean survival time were significant between trials with zero and eight parasites, trials with one and eight parasites, and trials with two and eight parasites ($P = 0.041$ for each; Kruskal-Wallis test coupled with Steel-Dwass test). In the medium size group, the values were 100% and 7.0 days for trials with zero or one isopods, 90% and 6.5 days, 50% and 4.9 days, and 30% and 3.9 days in trials involving two, four, or eight isopods, respectively, with a significant difference in mean survival time between trials with zero and eight parasites and trials with one and eight parasites ($P = 0.016$; Kruskal-Wallis test coupled with Steel-Dwass test). In the small size group, the respective values were 100% and 7.0 days for trials, 100% and 7.0 days for trials, 90% and 6.8 days, 60% and 5.4 days, and 10% and 2.1 days, with significant differences in mean survival time between trials with zero and eight isopods ($P = 0.0015$), trials with one and eight isopods ($P = 0.0015$), and trials with two and eight isopods ($P = 0.038$) (Kruskal-Wallis test coupled with Steel-Dwass test).

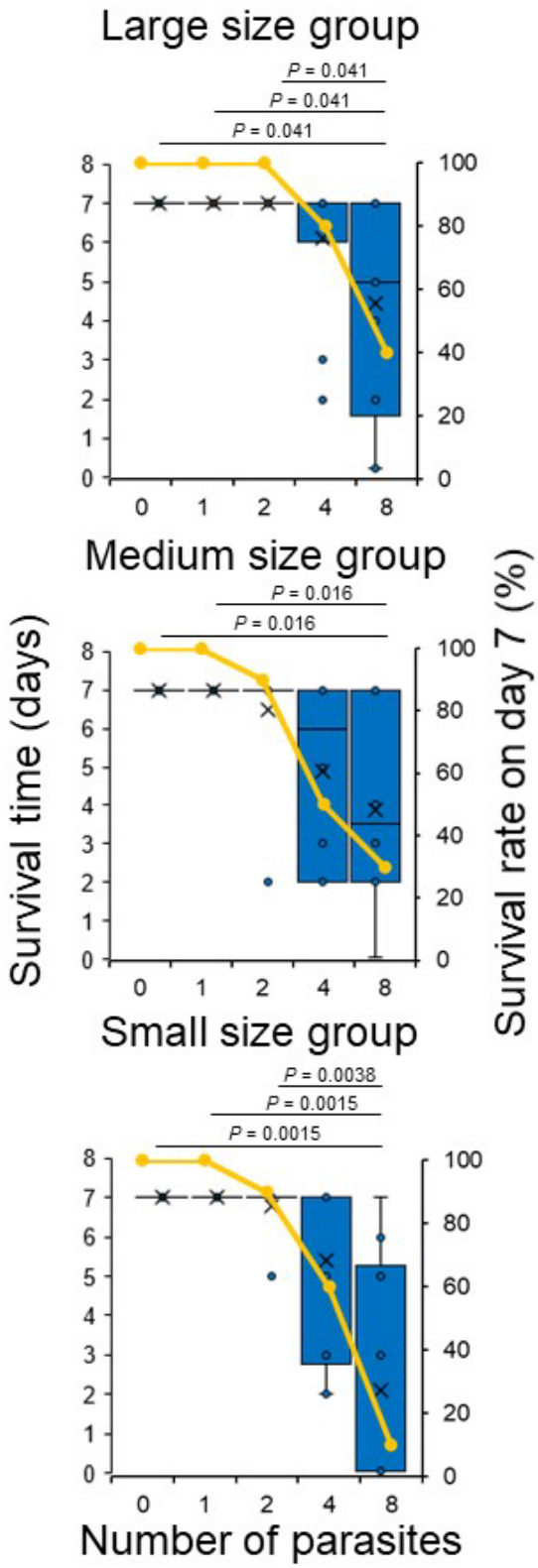

Fig. 8. Comparison of survival rates on day 7 (yellow line graph) and survival times (box plot) of host shrimp (*Palaemon paucidens*) of three size-classes experimentally exposed to different numbers of correspondingly sized parasitic isopods (*Tachaea chinensis*).

DISCUSSION

A comparison of the prevalence of *Tachaea chinensis* infesting *Palaemon paucidens* at our study site in Shimane Prefecture (80.0–90.2% at St. 1 in a semi-closed spillway and 45.0–55.0% at St. 2 in a small open stream) shows that the infection rate at St. 1 was extremely high compared to previous studies. The prevalence of *T. chinensis* on any species of host shrimp has never been reported to exceed 50% in the natural environment until now. In Japan, *P. paucidens* is the main host, with the reported prevalence of *T. chinensis* being 0–34.0% in the Tokyo Metropolis (Takahashi 2015), 43.5% in Ehime Prefecture (Imai et al. 2021), and 0–45.5% in Shiga Prefecture (Khalfan et al. 2022). As for other host shrimps in Japan, the prevalence of *T. chinensis* infestation was 0–18.8% on *Neocaridina* atyid shrimps in Shiga Prefecture (Ota 2019) and 11.8% on *Palaemon sinensis* (Sollaud, 1911) in Ehime Prefecture (Imai et al. 2021). In the laboratory, *T. chinensis* chose *P. paucidens* more than atyid shrimps (Maeda and Urabe 2022). They observed that *T. chinensis* initially on atyid shrimps easily moved to *P. paucidens*, in particular when *P. paucidens* was large. In China, reported prevalence of this isopod was 0.6–4.4% on *Palaemon carinicauda* Holthuis, 1950 in shrimp farms of Jiangsu Province (Xu et al. 2019). Another Chinese report revealed that *T. chinensis* parasitized over 80% of aquacultured *P. sinensis* in a rice field in Liaoning Province (Li et al. 2018; reported as *Palaemonetes sinensis*). The spillway and aquacultured rice field, which have high prevalence, may be similar in terms of semi-closed environment. Moreover, slow flow speed provides an excellent environment for the prevalence of parasites (Hallett and Bartholomew 2008).

The relative density of hosts and parasites at a given site is an important factor in studies of host-parasite species interactions, influencing both the prevalence and the intensity of infestation. (Wu et al. 2001; Mugabo et al. 2015; Buck et al. 2017; Stewart et al. 2022). Here we focus on intensity, starting with the positive correlation we observed between the number of parasites per shrimp (including zeros) and the prevalence of isopod infection in the field. It was showed that the intensity of ectoparasites on the freshwater climbing perch *Anabas testudineus* (Bloch, 1792) was related to their prevalence (Veronika et al. 2024). Such a relationship would be common in freshwater host/parasite systems.

Field surveys on the western shore of Lake Biwa, Shiga Prefecture, Japan (Ota 2019), recovered single parasites from 89.7% of infested *Neocaridina* spp. (305/340 cases), with double infestations on just 10.3% of these shrimp (35/340 cases). Similarly, surveys on the eastern shore of Lake Biwa (Khalfan et al. 2022) recovered single parasites on 98.3% of infested *Palaemon paucidens* (175/178 cases), with double infestations on just 1.7% of these shrimp (3/178 cases). These findings suggested that infested shrimp usually have one isopod in the natural environment. While, our unprecedented finds of up to nine parasites per host at St. 1 and a generally higher parasite load per shrimp there than at St. 2 thus require explanation. St. 1 is the spillway from an irrigation pond, and it alternates between existing as a continuous stream or a stagnant, isolated water body depending on the water level of the pond. At the time of collection on 2018, the spillway was cut off from the pond. The frequency and duration of periods of isolation were not recorded, but the cessation of downstream drift of shrimp from the pond during such periods may have the effect of lowering the number of available hosts in the spillway. If the shrimp trapped in the spillway are sufficiently few, more than one or two members of the resident population of *T. chinensis* might then be forced to attach to each host.

In the present study, usually just one or two parasitic isopods were found attached to a host's carapace, but in cases of heavier infestation (four to eight parasites), the limited surface area of the carapace apparently forced isopods to attach to other body regions, such as the abdomen. Shrimp hosting four or more isopods exhibited impaired walking and swimming behavior (own observations). In general, aquatic host organisms with attached ectoparasites require more energy to swim, feed, and avoid predation due to an increase in friction drag, which ultimately reduces their swimming speed (Binning et al. 2013; Li et al. 2020b). Predation pressure may lead to selection against individuals with more parasites. A negative effect of heavy infestation on host survivorship can, therefore, be expected. Indeed, in our study, the survival rate of *P. paucidens* through day 7 was 100% in trials with zero and one *T. chinensis*, but survivorship decreased as the number of parasites increased (90–100% with two parasites, 50–80% with four parasites, and 10–40% with eight parasites). In comparable studies, the survival rate through two weeks was 50% for the shrimp *Palaemon sinensis* when infested by one *T. chinensis* each (Li et al. 2018 2019; reported as

Palaemonetes sinensis) and 70% for another shrimp, *Macrobrachium nipponense* (De Haan, 1849), when infested by one isopod each for 15 days (Li et al. 2020a).

How does host mortality come about? Isopod parasites mainly feed on blood of the hosts after perforating the integument with their mandibles, causing stress, tissue damage, secondary infection, and mortality (Williams and Boyko 2012; Li et al. 2020a). An episode of mass mortality attributed in part to *T. chinensis* occurred in Okayama Prefecture, Japan, in a population of *P. paucidens* that had been captured in irrigation ponds and then reared in a concrete tank (Ueki et al. 1988). Up to three *T. chinensis* were found attached to the carapace or abdomen of the diseased shrimps, from which *Vibrio* sp., a pathogenic bacterium, was isolated. The isopod might have secreted antihemostatic, anti-inflammatory, and/or immunomodulatory compounds to facilitate bleeding during feeding while also reducing the effectiveness of the shrimp's antioxidant and immune defense systems against the invasion of pathogens (Li et al. 2019 2020b). In this way, infestation by multiple parasitic isopods could potentially accelerate the death of the host.

CONCLUSIONS

The present study showed that the number of ectoparasitic *Tachaea chinensis* per host shrimp was higher at St. 1 in a spillway than at St. 2 in a small stream farther downstream in both 2018 and 2019. This was probably due to the limited drift of new host shrimp from upstream by cut-offs of water flow and the higher parasitic-induced mortality of host shrimp at St. 1 compared to St. 2. This may be an exceptional situation reflecting periodic decreases in host density relative to the parasite population in the spillway, but efforts to prevent the invasion of *T. chinensis* into waterways outside its current range are nonetheless advised. Once such an invasion occurs, it may result in substantial economic losses for semi-closed freshwater shrimp farms there.

Acknowledgments: We would like to thank the associate editor Dr Hsi-Te Shih and three anonymous reviewers for their constructive comments. We also thank Keisuke Furuhata and Ryota Ikeo for their help in sample collection. This work was partially supported by the Grants-in-Aid for

Scientific Research from the Japan Society for Promotion of Science (KAKENHI), grant number 18K056688 and 220K05690.

Authors' contributions: Individual author contributions to the manuscript using the relevant roles as follows: research conceptualization, sample design and methodology; HS, investigation and data collection; AS, AMK, CO, DY, ST, TI and HS, data analysis and interpretation; AS, AMK and HS, funding provision; HS, roles/writing - original draft; writing - review & editing; AS, AMK, CO, DY, ST, TI and HS.

Competing interests: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Availability of data and materials: The datasets generated during the current study is available from the corresponding author on reasonable request.

Consent for publication: Not applicable.

Ethics approval consent to participate: Not applicable.

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