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Impact of sea spider parasitism on host clams: susceptibility and intensity-dependent mortality

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Nymphonella tapetis *(Pycnogonida, Ascorhynchidae) is an endoparasitic sea spider affecting bivalves. Recently, sea spiders have been found on a massive scale in the commercially important Manila clams (Veneridae,* Ruditapes philippinarum*) in Japan (Tokyo Bay). Simultaneously, mass mortality has occurred in this area. Local fishers assumed that this mass mortality was caused by the parasitic sea spider, despite the effect of the parasite and parasite intensity on the host being unknown. To evaluate the susceptibility of the Manila clam to sea spider infestation and the impact on mortality levels, we established six treatments at different infection intensities (density of newly hatched larvae of sea spiders) over a 6-month long laboratory experiment. We monitored mortality and three susceptibility indices (clearance rate, sand-burrowing speed and adductor muscle strength) under sufficient food conditions. Parasitization by sea spider affected clearance rate and sand-burrowing speed. The pattern of parasitic intensity effects on survival of Manila clam hosts was shown to be dependent on the levels of parasite numbers, i.e. clams with lower parasitic levels (total of <200 hatching larvae of sea spider given to a host) have a higher survival rate, and high mortality of host clams was shown in excessively higher parasitic densities (400–4000 individuals). Such pattern of parasitic effects on host survival might be one of the causes of mass mortality of Manila clams occurring in the field.*

Keywords: Clam fishery, endoparasite, host-parasite interaction, mortality, parasite intensity, sublethal impact

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INTRODUCTION

Parasites may regulate the population size of their host by causing high mortality (Møller, 2000; Altizer *et al.*, 2006) and by decreasing fecundity (Hudson *et al.*, 1998; Fredensborg *et al.*, 2005). Parasite-induced mortality has been investigated both theoretically and experimentally (Anderson & May, 1978; Adler & Kretzschmar, 1992). However interactive relationships between host and parasite such as symbiotic relationships are often complex and intensity-dependent factors that lead to host mortality are difficult to identify (May & Anderson, 1983; Tompkins & Begon, 1999).

Nymphonella tapetis (Ohshima, 1927) is a sea spider that is endoparasitic in bivalves, including the commercially important Manila clam *Ruditapes philippinarum* (Adams & Reeve, 1850), and had been found intermittently within the host clam since 1927 (Ohshima, 1927, 1933, 1935; Kikuchi, 1976; Ogawa & Matsuzaki, 1985). Until 2006, this parasite species had only rarely been found, and biological knowledge of it was limited, because its translucent body colour, behaviour of shallow sand-burrowing, small size and quite small population mean it is difficult to find.

In 2007, the species appeared suddenly and spread explosively on a massive scale infesting *R. philippinarium* and several other bivalves on the Banzu tidal flat in Tokyo Bay, Japan (Miyazaki *et al.*, 2010, 2015; Toba *et al.*, 2016). Simultaneously, mass mortality of bivalves was observed, leading to the shutdown of the local clam fishery. Understandably, it was assumed that mortality was due to the parasitism of the sea spiders. However, it has still not been confirmed that sea spider infection leads to high mortality of the clam (Ohshima, 1927, 1933, 1935; Kikuchi, 1976; Ogawa & Matsuzaki, 1985). If it can be established that parasitism by sea spiders is the cause of mortality within a

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population, then simultaneous outbreaks of sea spiders and mass mortality of host clams could be attributed to high levels of infestation. This study evaluated the susceptibility of the Manila clam *R. philippinarium* to sea spider infestation and the impact on mortality levels. The experiments were conducted under controlled laboratory conditions where environmental conditions and food supply could be maintained.

Adults of *N. tapetis* live freely on or just under the surface of sandy substrata. Adult males carry embryos on their ovigers until hatching, as is common in sea spiders. Hatched larvae enter the host bivalves and grow there, clinging to various soft parts and feeding on body fluid, probably until the maturation moult. After the first discovery of this species in 1926, there were occasional records from several places in Japan (Ohshima, 1927, 1933, 1935; Kikuchi, 1976; Ogawa & Matsuzaki, 1985; Miyazaki *et al.*, 2015). Reported host bivalve species for this sea spider include the Manila clam *R. philippinarum*, as well as the Common Orient clam *Meretrix lusoria*, duck clam *Mactra veneriformis*, razor clam *Solen strictus* and *Theora lata* (Kikuchi, 1976; Ogawa & Matsuzaki, 1985). All these bivalves occur commonly in Tokyo Bay but mass mortality supposedly due to sea spider parasitism was observed only in Manila clam (Miyazaki *et al.*, 2010; Toba *et al.*, 2016). In this study, therefore, we evaluate the impact of *N. tapetes* on populations of the Manila clam.

Based on population variation of adult sea spiders in the tidal flat sediments (e.g. Murauchi *et al.*, 2014), parasitic recruitment of sea spider into the Manila clam may occur at several times during the year (spring–autumn; Miyazaki *et al.*, 2010; Murauchi *et al.*, 2014; Yamada *et al.*, 2016b). Not all larval recruitment represents parasitic success, because the number of initial recruitment of newly hatched larvae (difficult to see with the naked eye and up to 42 individuals; Yoshinaga *et al.*, 2011) is higher than the number of immature parasites (visible with the naked eye and up to 15 individuals; Miyazaki *et al.*, 2010; Tomiyama *et al.*, 2016). It is presumed that a greater number of newly hatched larvae may parasitize a host clam until these observations of initial recruitment (Yoshinaga *et al.*, 2011). However, the infection dynamics of this sea spider (such as infection ratio, parasitic growth success rate until adulthood within host clams, and ratio of dropout in newly hatched larvae from host clam) are not well understood.

A sudden and significant presence of a newly observed species is commonly assumed to be indicative of it being an alien or recently introduced species. The same occurred in the case of *N. tapetis* in Tokyo Bay, although there has been dispute over whether *N. tapetis* is a native or alien species (Miyazaki *et al.*, 2010, 2015); many studies support its identification as an alien species because of its unexpected occurrence and fishery damage (Yoshinaga *et al.*, 2011; Chow *et al.*, 2012; Yoshinaga, 2012; Murauchi *et al.*, 2014).

MATERIALS AND METHODS

Laboratory experimental setting

The laboratory experiment was conducted in aquaria (height 30 cm, bottom area 800 cm^2), randomly placed in a running filtered seawater system (flow rate 0.5 l h^{-1}, salinity 27–32), with aeration under ambient light and temperature conditions (Appendix 1). Each aquarium contained one-third fine sand (depth 12 cm; median grain size: 180 μm), which was washed with filtered seawater and dried (at 98°C for >48 h). Sediment hardness in running filtered seawater was approximately 0.15 kPa (vane shear strength), suitable for sand-burrowing by the Manila clam (Sassa *et al.*, 2011).

Collection of host and parasite, and preparation for laboratory experiments

Experimental clams (N = 300, mean shell length 27.2 ± 1.2) were collected at particular sites in Tokyo Bay (35°24′N 139°54′E; northern parts of the Banzu tidal flat) and Lake Hamana (34°45′N 137°35′E) No sea spiders were detected in our preliminary observations from *c.* 300 clams captured at these sites.

In the laboratory, the clam specimens were acclimated in aquaria for >14 days with running aerated filter-cleaned seawater prior to the experiment, in order to remove the effect of the natural environment in the inhabited area (i.e. historical effect) and to ensure uniform conditions among the Manila clams (cf. Yamada *et al.*, 2016a). A diatom *Chaetoceros* spp. (cell diameter ~5–7 μm), was used to provide an algal diet. A sufficient feeding status of $5–10 \times 10^7$ cell·clam-g^{-1} day^{-1} (Toba & Miyama, 1991; Nakamura, 2004; Yamada *et al.*, 2016a) was offered for 12 h every day until the end of the laboratory experiments. The number of algal cells was counted in a Coulter counter (Coulter Z1, Beckman Coulter, Inc., Tokyo, Japan), and those with a particle diameter of >2.5 μm were regarded as *Chaetoceros* spp.

Newly hatched larvae were obtained from ovigerous males of *N. tapetis* for manipulating parasitism intensity (e.g. Ohshima, 1933, 1935). Ovigerous males (free-living) of *N. tapetis* carrying egg-mass sacks (~500 ind.) were collected from several sites at southern parts of the Banzu tidal flat in Tokyo Bay biweekly from May to August 2011. Ovigerous males of *N. tapetis* were kept in chambers (~20 ind. $aquarium^{-1}$) with running aerated filter-cleaned seawater and sufficient feeding status (*Chaetoceros* spp., $5–10 \times 10^7$ cell·sea spider-g^{-1}·day^{-1}), although the diet of free-living adults of this species is still not fully understood. As the sea spider larvae are not planktonic, newly hatched larvae were collected on the bottom every day. Hatched larvae were isolated in aerated chambers which were stored and kept in an incubator (15°C) under a dim light. Most eggs of a single male hatched within 7 days, and $\sim5 \times 10^7$ larvae (mean torso length, 46.2 ± 4.9 μm) were obtained from ~300 adult males. Before inducing parasitism into the Manila clam, dead larvae were removed. These operations were conducted four times for manipulating parasite from June to August 2011 (Appendix 1).

Experimental design and monitoring of mortality and susceptibility

In this experiment we examine the susceptibility of the host Manila clam to varying numbers of parasitic larvae. It has been reported that: (1) not all initial recruitment of newly hatched larvae translates into parasitic success because the number of initial recruitment of newly hatched larvae (up to 42 individualsYoshinaga *et al.*, 2011) is higher than the number of visible immature parasites (up to 15 individuals; Miyazaki *et al.*, 2010; Tomiyama *et al.*, 2016); (2) a greater

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number of newly hatched larvae may parasitize a host clam until observations of initial recruitment (Yoshinaga *et al.*, 2011); and (3) parasitic recruitment of sea spider into the Manila clam may occur at several times during a year (spring–autumn: Murauchi *et al.*, 2014; Yamada *et al.*, 2016b). Based on these observations, we established six levels of parasite number for study: (1) control (*CL*: parasite-free), (2) Level 1 (*L1*; 10 parasite larvae per host × 4 times), (3) *L2* (50 parasite larvae × 4 times), (4) *L3* (100 parasite larvae × 4 times), (5) *L4* (500 parasite larvae × 4 times), and (6) *L5* (1,000 parasite larvae × 4 times) (Appendix 1). Miyazaki *et al.* (2010), Yoshinaga *et al.* (2011) and Murauchi *et al.* (2014) found that the naturally occurring number of parasites in each bivalve was up to ~50 individuals. Based on this estimate, we established the number of parasites in *L2* as natural parasite density. Thus, *L3–6* would be extreme parasite densities. For each of the six levels, 50 individuals of the clam were introduced into each infection level (*L*) aquarium, giving a density of approximately 350 ind. m^{-2}, very similar to natural densities in the tidal flats of Japan (Yamada *et al.*, 2014; Tomiyama *et al.*, 2016).

Parasite infection was conducted by directly introducing 100 µl of seawater including newly hatched larvae into the Manila clam by pipette (Appendix 1). 100 µl of filtered seawater with a uniform density of newly hatched larvae and only seawater for the control (*CL*) were poured into the Manila clam using a tapered pipette by shell opening slightly (<0.5 mm width). The survival rate of the parasites was measured by counting the number of free-living adults in each aquarium (see Results).

In the Banzu tidal flat, *N. tapetis* has a continuous reproductive season from spring to autumn (when temperatures are >15°C, Appendix 2; Miyazaki *et al.*, 2010; Yoshinaga *et al.*, 2011; Yamada *et al.*, 2016b). We therefore monitored mortality and three mortality susceptibility indices (i.e. susceptibility leading to the mortality) of the host in ambient environmental conditions during this period (June–November) in the laboratory.

Clam mortality, and the presence of free-living adults to assess the success of induced parasitism, were monitored daily. With regard to mortality susceptibility indices, we measured monthly clearance rates to represent feeding activity, and sand-burrowing activity and adductor muscle strength to represent physical capability (see below). A total of 3–5 individual clams were randomly selected from each of the six levels (*L*), and the three mortality susceptibility indices were measured; this was done to avoid continuous measurement of a specific individual. After measurement, these individuals were placed back into their respective aquarium. Considering the experimental conditions, the mortality susceptibility indices determined by this indirect method may not be representative of the true filtration rate of the clam in natural conditions. When taking into account the simplicity of our study, these values could potentially be used in comparative studies.

Measurement of susceptibility

Feeding activity of the clams as a mortality susceptibility index was assessed in terms of clearance rate (*CR*), as per Nakamura (2004):

$$CR = (V/t_s) \cdot ln(C_n/C_{n+1}) - CR^* \quad (1)$$

where V is the volume of the medium in the chamber, C_n and C_{n+1} are the algal concentration (*Chaetoceros* spp. cells ml^{-1}, measured by Coulter Z1) at the start (time = 0) and at time t_s, respectively, and CR^* is the apparent clearance rate due to settling of algae on the floor of the chamber. CR^* was estimated by monitoring changes in algal concentration (cell ml^{-1}) in a chamber without clams (control):

$$CR^* = (V/t_s) \cdot ln(C_o^*/C_1^*), \quad (2)$$

where Co^* and $C1^*$ are algal concentration (cell ml^{-1}) in the control at the start and at time t_s, respectively. The chamber measurement for clearance rate was conducted in ambient conditions with gentle aeration for about 30 min.

The physical capability of the clams as a mortality susceptibility index was assessed from sand-burrowing speed (mm s^{-1}) and adductor muscle strength (g) (i.e. the shell-closing strength). In the measurement of sand-burrowing speed, clams were reared in covered chambers (inner diameter 8.5 cm; height 30.0 cm) containing washed and dried sand (median grain size: 180 µm and sand depth ~15 cm) and ~280 ml of rearing water. The Manila clam, acclimatized for 1 day, was placed on the surface of the sand. Burrowing times, from touching the sediment surface with its foot to burying the tip of the shell (according to Sassa *et al.*, 2011), were recorded for 3–5 randomly selected individuals. When the clam did not move for >30 min, the sand-burrowing experiment was ceased and postponed to another day, again using randomly selected clams.

A spatula-shaped pressure sensor (SsPS, Sensor Net Co. Ltd, Yamagata Prefecture, Japan; 5 mm width and <0.5 mm height) was used for measuring adductor muscle strength (cf. Tomiyama *et al.*, 2016). The head of the SsPS was inserted (2–3 mm) between the valves into the half-length of the clam shell, with these preconditioned for >1 h in chambers containing sand (depth ~15 cm) with aerated rearing water. Pressures were continuously recorded every second from 5 to 60 s after insertion (55 datasets per observation) using an instrumentation amplifier (WGI-400, KYOWA Co. Ltd, Japan). The maximum pressure value was expressed as adductor muscle strength for comparison amongst the control and *L1-L5* infection level specimens. Although clams were opened slightly after this operation, we confirmed in the initial procedure that this manipulation did not adversely affect the clams.

Statistical analysis

Differences in mortality among parasite infection levels were evaluated using Cox proportional-hazards regression analysis (Cox, 1972). The analysis included levels and experimental date (nested within levels) as explanatory variables. Differences in the occurrence of sea spiders and susceptibility among levels (aquaria) were evaluated using a generalized linear mixed model (*R* ver. 3.0.2 [http://cran.r-project.org/]), because pseudoreplication occurs in randomly chosen measurement terms (e.g. Yamada *et al.*, 2014). We modelled parasite intensity (*CL, L1–5*) as a fixed effect and experimental data as a random effect (family = Poisson). Modelling measurement terms as a random effect controlled for pseudoreplication and accounted for the variance between particular terms. Variance components of random effects were estimated using the restricted maximum likelihood method. Tukey post

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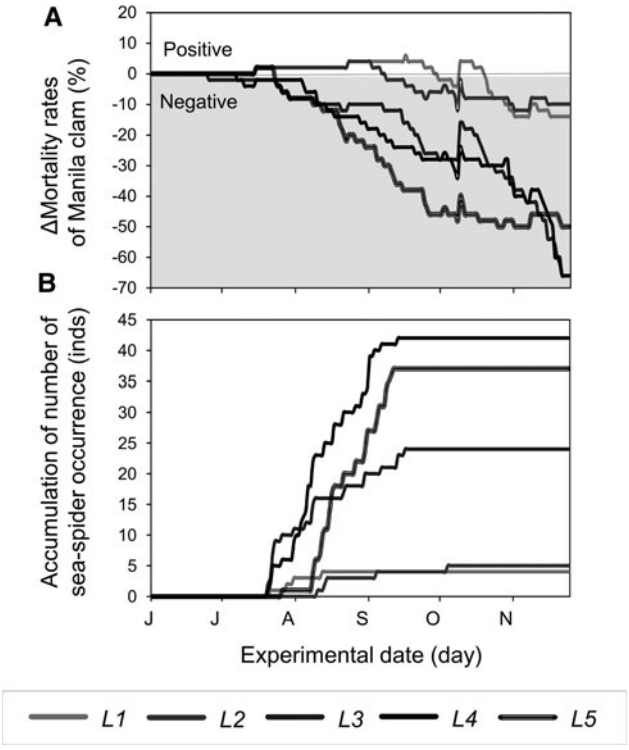

Fig. 1. Temporal changes in (A) Δ mortality rate (by control) of the host Manila clam *Ruditapes philippinarum* with levels (*L1–5*) of parasite number, and (B) accumulation of the number of free-living adult sea spiders, *Nymphonella tapetis*, during the experimental period (June–December).

hoc tests were carried out to determine which time and treatments differed.

RESULTS

Mortalities (%) were significantly different among levels of parasite number ($df = 5$, $\beta = 0.608$, Wald statistics = 25.78, $P < 0.001$, Figure 1A). Mortalities in *L1* and *L2* were not significantly different from controls (*L1*: $z = 1.12$, $P = 0.2611$; *L2*: $z = 1.08$, $P = 0.2784$), while mortalities in *L3–5* were significantly higher (*L3*: $z = 3.48$, $P < 0.001$; *L4*: $z = 3.67$, $P < 0.001$; *L5*: $z = 3.25$, $P = 0.001$). The occurrence pattern of adult sea spiders in experimental aquaria (ind. aquarium^{-1}) was also significantly different among levels ($df = 4$, $\chi^2 = 35.309$, $P < 0.001$, Figure 1B). Specifically, the number of occurrences in *L1* and *L2* were significantly lower than in *L4* and *L5*, with *L3* intermediate among them.

The three mortality susceptibility indices showed typical seasonal changes, i.e. during higher temperature periods (summer: July–August), the clearance rate and sand-burrowing speed were higher, and adductor muscle strength (g) was lower (Figure 2). The clearance rate and sand-burrowing speed were significantly different among levels (clearance rate: $df = 5$, $\chi^2 = 12.223$, $P = 0.032$; sand-burrowing speed: $df = 5$, $\chi^2 = 12.355$, $P = 0.030$, Figure 2). However, although significant differences among levels were shown in September and November for clearance rates, and in July, September and November for sand-burrowing speed, a decreasing trend along level gradients was not evident in these months (Figure 2). Adductor muscle strength did not differ significantly among levels ($df = 5$, $\chi^2 = 6.983$, $P = 0.222$) (Figure 2).

In order to evaluate the effect on the host, we show differences in susceptibility between each parasite level (*L*) and *CL* (as Δ susceptibility) in Figure 3. Because *L* should be lower than *CL* due to effect of parasite, Δ susceptibility would show negative changes. However, there was no clear trend in temporal changes in Δ clearance rate, Δ sand-burrowing speed and Δ adductor muscle strength (Figure 3). The negative relationships between levels and mortality susceptibility indices were not clear, although negative effects were often observed in *L4* and *L5* for Δ clearance rate, *L3* and *L4* for Δ sand-burrowing speed, and *L2–4* of Δ adductor muscle strength; nevertheless, a clear decreasing gradient across levels was not observed.

DISCUSSION

Parasite number corresponded to a pattern of variation in temporal mortality along levels (Figure 1A). The mortality of Manila clams can be correlated with the level of parasitic infection by the sea spider. Notably, the pattern of parasitic intensity effects on survival of Manila clam hosts was shown to be dependent on the levels of parasite numbers. In other words, clams with lower parasitic levels have a higher survival rate. This study is the first to report a potential correlation between the mortality of this commercial fishery species and parasitic infection by sea spiders.

The results of occurrence of adult sea spider in experimental aquaria indicate that introduction of parasitism in clams was accomplished in this study (Figure 1B). This result provides valuable information on the duration of the stage from newly hatched larva to sea spider adult, shown to be 1–1.5 months, based on the first occurrence of free-living adults 50 days from first infection in June (Figure 1B). This information is helpful in understanding the life cycle of the sea spider (e.g. Murauchi *et al.*, 2014; Yamada *et al.*, 2016b). On the other hand, occurrence number of adult sea spider from clam hosts were low, compared with numbers of initial-parasite introduction to an excessively high number (<5% of total number of newly hatched larvae in initial-parasite introduction). There might be two reasons for this: (1) the early parasitic success ratio by newly hatched larvae of sea spiders is quite low; and (2) parasites were successful only during limited periods (e.g. if one-time induced parasitism manipulation (e.g. only on 11 July) was successful (Appendix 1), parasitic success would be 20%). However, we could not confirm early parasitic success by newly hatched larvae of sea spiders due to technical problems caused by the necessity of opening (killing) clams to confirm early parasitic success.

In this study, we demonstrated that survival and susceptibility of host clams were dependent on the levels of parasite numbers. However, we also detected that significantly higher mortality of host clams was shown in cases of excessively higher parasitic densities (*L3–5*), and cases of weak influence of the parasitic sea spider on host susceptibility were also shown. These results suggest that mere presence of the parasitic sea spider (i.e. normal level of parasite) may not always be concerned with survival and susceptibility of host clams. In fact, the results of our experiment indicate that clams with lower parasitic levels (*L1–2*) had a high survival rate. This is consistent with results obtained in other clam parasite

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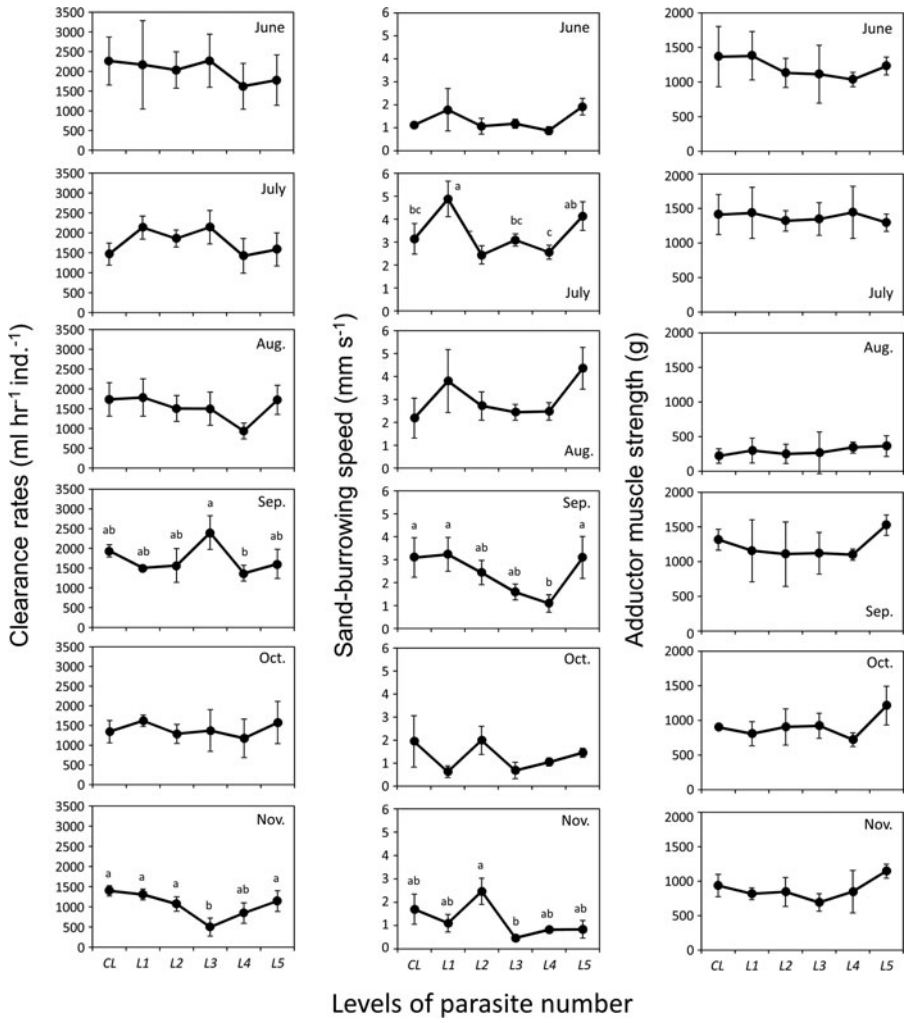

Fig. 2. Variation of the three mortality susceptibility indices (clearance rate, sand-burrowing speed and adductor muscle strength) of the host Manila clam *Ruditapes philippinarum* parasitized by the sea spider *Nymphonella tapetis* at control (*CL*) and at each Level (*L1–5*) of parasite number during each experimental month (June–December).

species (e.g. Yoshinaga *et al.*, 2010; Waki & Yoshinaga, 2013). Generally, the number of endoparasitoid species is quite low among the total fauna (e.g. May & Anderson, 1983; Tompkins & Begon, 1999). This is because parasites that kill their hosts result in the parasite's own death, which is quite an inefficient biological interaction for population maintenance of parasite species from both ecological and evolutionary perspectives (Anderson & May, 1978; Adler & Kretzschmar, 1992). In the case of *N. tapetis*, although there is a perception that sea spiders have a strong influence on Manila clam mortality (Chow *et al.*, 2012; Yoshinaga, 2012), it is possible that the mass mortality of the clam cannot be solely attributed to *N. tapetis*.

This suggestion that the mass mortality of clams is not caused only by the sea spider but also by other factors is also supported by our results of susceptibility variations. Although clearance rate and sand-burrowing speed were significantly different among treatments (Figure 2), a clear decreasing trend across parasite levels was not evident for any delta (Δ) in susceptibility indices (Figure 3). This result supports that parasitization by sea spiders may not always have a negative effect on host susceptibility. In this laboratory experiment, we offered sufficient feeding states and a stable water environment to host clams, suggesting that such experimental conditions may lead to less infection effect on susceptibility variations.

In order to confirm effect in the results of this study (i.e. less infection effect on mortality and/or susceptibility of host clam) with such a stable condition for host clam in laboratory experiment, we established additional treatments under no food conditions in *L2*, and monitored the mortality of host clams. As a result, the number of infected clams in this treatment decreased exponentially, and all infected clam individuals were dead within 34 days (data not shown). On the other hand, infected clam individuals in *L2* treatments with sufficient food survived (not all dead) until the end of the experiment of this study (~6 months; Figure 1A). These results indicate a notable effect of interaction between parasitic infection and lack of food on host clams, supporting that mass mortality of host Manila clam in the field (Tokyo Bay) may not be caused solely by parasitic infection of sea spiders, and might possibly be due to interactive effects between the parasitic infection and other factors, such as lack of food and severe environmental conditions (e.g. hypoxia and low salinity; Wakita *et al.*, 2014; Yamada *et al.*, 2016a).

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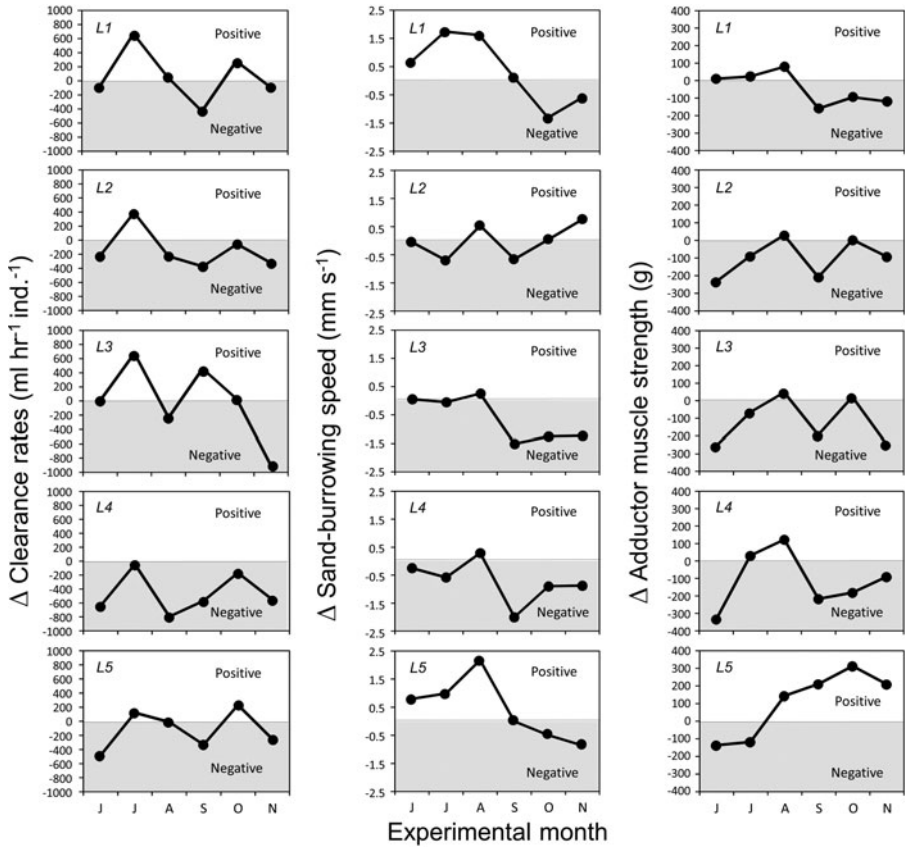

Fig. 3. Temporal changes in the three Δ mortality susceptibility indices (Δ clearance rate, Δ sand-burrowing speed, and Δ adductor muscle strength, against control) of host Manila clam *Ruditapes philippinarum* parasitized by the sea spider *Nymphonella tapetis* at each level (*L1–5*) of parasite number during each experimental month (June–December).

The result of a lower infection effect in normal parasitic levels on mortality and/or susceptibility of host clam also imply that the Manila clam as host of parasitic sea spider might have some strategies to avoid and/or reduce the effect of infection (e.g. May & Anderson, 1983; Tompkins & Begon, 1999). If so, the fact that occurrences of parasitic sea spiders on the Manila clam have been recorded for many decades (since 1926; Ohshima 1927, 1933, 1935) suggests that the Manila clam might have historical experience (e.g. a selective advantage). This supports that *N. tapetis* might ecologically be regarded as a native species, although many studies have identified *N. tapetis* as an alien species because of its unexpected occurrence and fishery damage (Yoshinaga *et al.*, 2011; Chow *et al.*, 2012; Yoshinaga, 2012, Murauchi *et al.*, 2014).

CONCLUSION

We evaluated the influence of the parasitic sea spider *N. tapetis* on the host Manila clam *R. philippinarum*, focusing in particular on the survival and susceptibility of the host by manipulating parasite number. Mortality of the Manila clam depended on the levels of parasite numbers, but pattern of mortality was significantly different between the levels of parasite numbers (*L1–2* and *L3–5*). Susceptibility of Manila clam was affected by the parasitization but not always. These results suggest that mere presence of the parasitic sea spider (i.e. normal level of parasite) may not be concerned with survival and susceptibility of host clams, and is therefore possibly situation-dependent. For example, feeding state and environmental variations might influence infection effects. Our results also suggest that such interactive effects of infection and environmental variations on susceptibility of Manila clams would provide an important future perspective for evaluation of infection effects (Møller, 2000; Altizer *et al.*, 2006).

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REFERENCES

Adler F.R. and Kretzschmar M. (1992) Aggregation and stability in parasite-host models. *Parasitology* 104, 199–205.

Altizer S., Dobson A.P., Hosseini P., Hudson P., Pascual M. and Rohani P. (2006) Seasonality and the dynamics of infectious disease. *Ecology Letters* 9, 467–484.

Anderson R.M. and May R.M. (1978) Regulation and stability of hostparasite population interactions, I: regulatory processes. *Journal of Animal Ecology* 47, 219–247.

Chow S., Niwa K., Okamoto S., Murauchi Y., Hirai R., Hibino M., Wakui K., Tomiyama T., Kobayashi Y., Toba M. and Kano Y. (2012) Genetic diversity in three local populations of the parasitic sea spider *Nymphonella tapetis* and taxonomic position of the species. *Nippon Suisan Gakkaishi* 78, 895–902. [In Japanese with English abstract].

Cox D.R. (1972) Regression models and life tables. *Journal of the Royal Statistical Society B* 34, 187–202.

Fredensborg B.L., Mouritsen K.N. and Poulin R. (2005) Impact of trematodes on host survival and population density in the intertidal gastropod *Zeacumantus subcarinatus*. *Marine Ecology Progress Series* 290, 109–117.

Hudson P.J., Dobson A.P. and Newborn D. (1998) Prevention of population cycles by parasite removal. *Science* 282, 2256–2258.

Kikuchi T. (1976) Rediscovery of the bivalve-infesting pycnogonid, *Nymphonella tapetis* Ohshima 1927, in Amakusa. *Publications from the Amakusa Marine Biological Laboratory* 4, 51–56.

May R.M. and Anderson R.M. (1983) Epidemiology and genetics in the coevolution of parasites and hosts. *Proceedings of the Royal Society, B* 219, 281–313.

Miyazaki K., Kobayashi Y., Toba M. and Tsuchiya H. (2010) Biology of *Nymphonella tapetis* Ohshima, 1927, a harmful pycnogonid endoparasitic on the commercial bivalve, *Ruditapes philippinarum*. *Proceedings of the Japanese Society of Systematic Zoology* 28, 45–54. [In Japanese with English abstract]

Miyazaki K., Tomiyama T., Yamada K. and Tamaoki M. (2015) 18s analysis on the taxonomic position of an endoparasitic pycnogonid *Nymphonella tapetis* (Arthropoda, Pycnogonida) in the family Ascorhynchidae. *Journal of Crustacean Biology* 35, 491–494.

Møller A.P. (2000) Survival and reproductive rate of mites in relation to resistance of their barn swallow hosts. *Oecologia* 124, 351–357.

Murauchi Y., Okamoto S., Hirai A., Miyazaki D., Yamamoto N., Hibino M., Kawamura K., Harada M., Okamura Y. and Hattori K. (2014) Seasonal dynamics of the parasitic larvae free-living adults of the sea spider *Nymphonella tapetis* infecting the Manila clam *Ruditapes phillipinarum* in the eastern coast of China peninsula, and the effect of water temperature on the infection levels. *Aquaculture Science* 62, 183–190. [In Japanese with English abstract]

Nakamura Y. (2004) Suspension feeding and growth of juvenile Manila clam *Ruditapes philippinarum* reared in the laboratory. *Fisheries Science* 70, 215–222.

Ogawa K. and Matsuzaki K. (1985) Discovery of bivalve-infesting Pycnogonida, *Nymphonella tapetis*, in a new host, *Hiatella orientalis*. *Zoological Science* 2, 583–589.

Ohshima H. (1927) *Nymphonella tapetis*, ng, n. sp., a pycnogon parasitic in a bivalve. *Annotationes Zoologicæ Japonenses* 11, 257–263.

Ohshima H. (1933) The adult of the bivalve-infesting pycnogonid, *Nymphonella tapetis* Ohshima. *Annotationes Zoologicæ Japonenses* 14, 53–60.

Ohshima H. (1935) A further note on *Nymphonella tapetis*: the egg-carrying mature male (Eurycydidae: Pantopoda). *Annotationes Zoologicæ Japonenses* 15, 95–102.

Sassa S., Watabe Y., Yang S. and Kuwae T. (2011) Burrowing criteria and burrowing mode adjustment in bivalves to varying geoenvironmental conditions in intertidal flats and beaches. *PLoS ONE* 6, e25041. http://dx.doi.org/10.1371/journal.pone.0025041.

Toba M., Kobayashi Y., Kakino J., Yamakawa H., Ishii R. and Okamoto R. (2016) Stocks and fisheries of asari in Japan. *Bulletin of Japan Fisheries Research and Education Agency* 42, 9–21.

Toba M. and Miyama Y. (1991) Gonadal development and spawning induction in artificially conditioned manila clams *Ruditapes philippinarum*. *Nippon Suisan Gakkaishi* 57, 1269–1275. [In Japanese]

Tomiyama T., Yamada K., Wakui K., Tamaoki M. and Miyazaki K. (2016) Impact of sea spider parasitism on host clams: relationship between burial patterns and parasite loads, somatic condition, and survival of host. *Hydrobiologia* 770, 15–26.

Tompkins D.M. and Begon M.E. (1999) Parasites can regulate wildlife populations. *Parasitology Today* 15, 311–313.

Waki T. and Yoshinaga T. (2013) Experimental challenges of juvenile and adult Manila clams with the protozoan *Perkinsus olseni* at different temperatures. *Fisheries Science* 79, 779–786.

Wakita K., Yamakita T., Yamada K., Yagi N. and Kurokura H. (2014) An evaluation of Tokyo Bay as a marine space for *Porphyra* spp. farming: evaluating marine spaces through quantitative examination of water environmental variables across categorized marine spaces, making use of multiple institutional data. *The Japanese Society of Fisheries Science* 80, 689–701. [In Japanese with English abstract]

Yamada K., Miyamoto Y., Fujii C., Yamaguchi K. and Hamaguchi M. (2014) Vertical zonation and aggregated distribution of the Manila clam on subtidal sandy flats in a coastal brackish lagoon along Sea of Japan. *Marine Ecology* 35, 408–418.

Yamada K., Miyamoto Y., Nakano T. and Okamura K. (2016a) Inter- and intraspecific variation in anoxic survival among three bivalve species in intertidal and subtidal areas along the coast of Japan. *Plankton and Benthos Research* 11, 49–56.

Yamada K., Miyazaki K., Chow S., Yamamoto T., Tomiyama T., Yoshinaga T., Miyama Y., Tamaoki M. and Toba M. (2016b) Life history and distribution of *Nymphonella tapetis*, a sea spider parasitic on the Manila clam, *Ruditapes philippinarum* in Tokyo Bay, Japan. *Bulletin of Japan Fisheries Research and Education Agency* 42, 94–95.

Yoshinaga T. (2012) Works toward development of countermeasures against fish and shellfish parasitic diseases. *Nippon Suisan Gakkaishi* 78, 384–387. [In Japanese]

Yoshinaga T., Kobayashi Y., Toba M. and Miyama Y. (2011) Development of a sensitive method for the detection of young larvae of the parasitic pycnogonid *Nymphonella tapetis* in Manila clam *Ruditapes philippinarum*. *Fish Pathology* 46, 38–41.

and

Yoshinaga T., Watanabe S., Waki T., Aoki S. and Ogawa K. (2010) Influence of *Perkinsus* infection on the physiology and behavior of adult Manila clam *Ruditapes philippinarumi*. *Fish Pathology* 45, 151–157. [In Japanese with English abstract].

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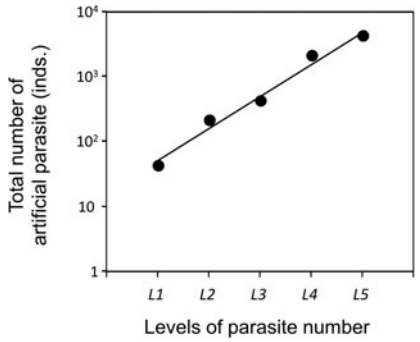
APPENDIX 1

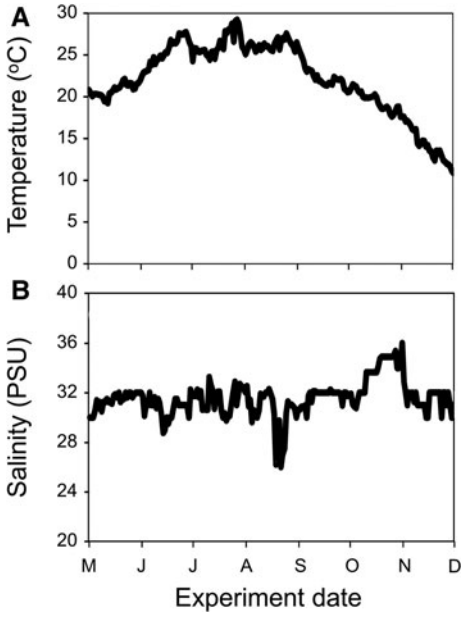
Appendix 1A. Date and number of induced parasites (newly hatched larvae) of the sea spider on the host Manila clam in experiments.

Treatments	Date				Total
	30-May	17-Jun	11-Jul	12-Aug	
Control (*CL*)	0	0	0	0	0
Level 1 (*L1*)	10	10	10	10	40
Level 2 (*L2*)	50	50	50	50	200
Level 3 (*L3*)	100	100	100	100	400
Level 4 (*L4*)	500	500	500	500	2000
Level 5 (*L5*)	1000	1000	1000	1000	4000



Appendix 1B. Relationship between levels (*L1–5*) of parasite number and total number of induced parasites (newly hatched larvae) of sea spider in the Manila clam.

APPENDIX 2



Appendix 2A. Temporal changes in (A) mean temperature (°C) and (B) mean salinity of aquaria on experimental dates (May–December 2011).

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