

## Effects of Morphological Changes Induced by the Rhizocephalan Parasite *Polyascus polygenea* on Predation Risk of the Asian Shore Crab *Hemigrapsus sanguineus*

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Parasites can significantly influence ecological communities by altering the traits of their hosts. Rhizocephalans, a group closely related to thoracican barnacles within Cirripedia, are common in marine ecosystems and profoundly impact their hosts, most notably by inducing reproductive castration. However, their influence on other host traits, particularly those related to predator defense, remains underexplored. The rhizocephalan *Polyascus polygenea* modifies the length of the cheliped propodus in the host crab *Hemigrapsus sanguineus*. To determine whether parasitized crabs are more vulnerable to predation, we conducted field tethering experiments comparing parasitized and unparasitized individuals. The results showed that parasitism itself did not directly increase the likelihood of crabs being lost (presumably due to predation) in either sex. However, structural equation modeling revealed that in parasitized males, a reduced cheliped propodus length indirectly increased predation risk. In females, parasitism reduced carapace width, which in turn shortened the cheliped propodus and increased vulnerability to predation. These findings demonstrate that *Polyascus* infection heightens predation risk through non-consumptive effects by inducing host morphological changes.

**Keywords:** Predator-host-parasite interaction, Predation of the host crab, Sacculinid, Morphological change, Cheliped propodus length

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## BACKGROUND

Parasites play a key role in shaping marine ecosystems by altering the traits of their hosts (Kuris 1974; Kuris and Lafferty 1992). Rhizocephalans (Thecostraca: Cirripedia) parasitize a variety of crustaceans, including hermit crabs (Yoshida et al. 2014; Jung et al. 2019; Kajimoto et al. 2022), crabs (Lützen et al. 2018; Toyota et al. 2023), and shrimps (Lützen et al. 2016). These parasites are particularly notable for causing parasitic castration, rendering hosts reproductively inactive (Hines et al. 1997). Rhizocephalans are highly adapted parasites, with adult females developing root-like internal structures (internae) for nutrient absorption and external reproductive structures (externae). Males are drastically reduced in size and live inside females (Høeg 1995a b; Høeg and Lützen 1995; Høeg et al. 2019 2020).

Several species, particularly in the family Sacculinidae, induce feminization in male hosts, altering secondary sexual traits such as abdominal morphology, chela size, and copulatory appendages (Alvarez and Calderon 1996; Kristensen et al. 2012; Waiho et al. 2017; Toyota et al. 2023). A well-documented example is the transformation of a male's narrow, semicircular abdomen into a broader, more female-like shape, especially in brachyuran crabs. This adaptation allows parasitized males to accommodate larger externae, potentially increasing parasite reproductive success (Rees and Glenner 2014; Nagler et al. 2017; Mouritsen et al. 2018). Other morphological changes include reduced chela size, altered copulatory structures (Hartnoll 1962; Kristensen et al. 2012; Toyota et al. 2023), and changes in pleopod number (Kristensen et al. 2012).

Although such parasite-induced morphological changes have been well documented, their implications for host survival are not well understood. In particular, no studies have investigated how changes in chela size—a key component of anti-predator defense—might affect host vulnerability to predation. This study aims to address this gap by examining whether parasitism by *Polyascus polygenea* increases the susceptibility of *Hemigrapsus sanguineus* to predation through a reduction in cheliped propodus length. Field experiments were conducted along the coast of Osaka, Japan.

## MATERIALS AND METHODS

## Ethical statement

No specific permissions were required for this study, as it did not involve endangered or protected invertebrate species in Japan. Vertebrates, humans, or human-derived tissues were not used. All animal experiments were conducted in compliance with the ARRIVE guidelines (Percie du Sert et al. 2020).

## Sample collection and rearing of host crabs

The prevalence of rhizocephalan infection is known to vary seasonally, although no previous studies have documented such variation at this particular field site (Osaka, Japan). To obtain a sufficient number of host crabs for the field predation assay, we conducted sampling over several months. Between July and November 2021 and April and August 2022, 10 to 30 individuals of the Asian shore crab *Hemigrapsus sanguineus* were collected by hand at least once per month from the rocky coastline of Shin Town, Hannan City, Osaka, Japan (34.213°N, 135.135°E). For species identification of sacculinids, an additional eight parasitized individuals were collected from the same site in July 2025.

While waiting to accumulate enough individuals for the field predation assay, the collected crabs were temporarily maintained in the laboratory. Each crab was housed individually in a round plastic container (7.2 cm diameter × 16.7 cm height) filled with 150 mL of artificial seawater (Gex, Osaka, Japan) and maintained at  $25 \pm 1^\circ\text{C}$  under fluorescent room lighting. The seawater was replaced every 3 days, and crabs were fed approximately 30 mg of commercial crayfish feed (“Kyozaï zarigani no esa,” Kyorin, Hyogo, Japan) with each water change.

## Species identification based on morphological characteristics and cytochrome *c* oxidase subunit 1 (*COI*) sequencing

A portion of the externae obtained from eight sacculinids parasitizing *H. sanguineus*, which had been preserved in absolute ethanol at room temperature, was excised for DNA extraction. Genomic DNA was extracted from the externae using DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer’s protocol. A fragment of the mitochondrial cytochrome oxidase subunit I (*COI*) gene from sacculinids was amplified by PCR using the primer set L2020S (5'-GAC CCT GCG GGW GGR GGR GAT CC-3') and H2619 (5'-GGT ATW CCW GCK AGW CCT AAG AAA TGT TG-3'), as previously described by Toyota et al. (2023). The PCR product was sequenced using the Eurofins sequencing service (Eurofins Genomics, Tokyo, Japan). The eight

sequences of the sacculinids obtained in this study were deposited in the DNA Data Bank of Japan (DDBJ) under the accession numbers LC888069–LC888076.

### **Measurements of morphological traits**

The sex of each crab was determined by the presence of female pleopods or male gonopods. We then recorded the presence of a mature sacculinid externa on the abdomen. Carapace width (CW) and the length of the right and left cheliped propodus (CPL) were measured using a digital caliper. The average of the right and left CPL (Ave CPL) was calculated for each individual, based on the assumption that both claws are used defensively against predators.

### **Field predation assay**

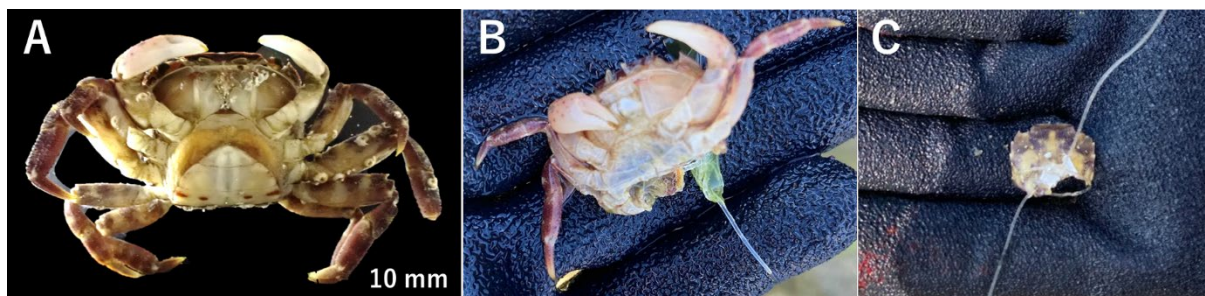
Prolonged holding in the laboratory resulted in increased mortality of host crabs, prompting us to divide the experiments into multiple smaller trials rather than conducting them all at once. Due to the limited number of individuals that could be collected per sampling day, it was difficult to fully standardize crab size among experimental groups. Given these constraints, we determined that using two crabs per category per tethering trial was the most practical strategy for maintaining consistent experimental conditions.

Two crabs with carapace widths between 10 and 35 mm were randomly selected from each of the following groups: parasitized males, unparasitized males, parasitized females, and unparasitized females. The dorsal carapace of each crab was dried with a towel, and a 30-cm monofilament fishing line was affixed using a droplet of quick-drying glue. To accelerate bonding, 1 mL seawater was applied to the glue. The other end of the line was tied to a ring attached to a small rock (~20 cm diameter), allowing the crab to hide beneath the rock but limiting its range of movement. Eight tethered crabs (two per group) were randomly spaced at least 1 m apart in the lower intertidal zone. Crab loss (presumed predation) was monitored daily for three consecutive days.

To control for non-predatory losses, eight crabs were similarly prepared and placed inside mesh cages (29.5 cm width × 23.3 cm depth × 17.0 cm height; 2.31 mm mesh size). These cages allowed for water flow but prevented predator access. A total of 12 replicates were conducted at the same site between September–November 2021 and May–September 2022, yielding 24 individuals per crab category.

### **Statistical analysis**

The data from eight host crabs that were presumed to have molted during the experiment—one unparasitized male, two parasitized males, one unparasitized female, and four parasitized females (identified by the presence of intact exuviae with no feeding marks; see Fig. 1)—were excluded from all analyses.



**Fig. 1.** Examples of (A) parasitized, (B) molted, and (C) lost (presumably preyed) host crabs *Hemigrapsus sanguineus*.

To test whether sacculinid infection directly affected the likelihood of crab loss, generalized linear models (GLMs) with a binomial distribution and logit link function were applied separately for males and females. In this model, the presence of sacculinid externa was the explanatory variable, and crab fate (intact or lost) was the dependent variable. Analyses were conducted using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). To examine whether CW or Ave CPL directly influenced crab loss, additional GLMs (binomial, logit link) were performed separately for each sex, with CW or Ave CPL as the explanatory variable.

The effect of rhizocephalan infection on relative Ave CPL was analyzed by testing for equality of regression slopes using CW as a covariate. When no significant interactions were detected, an ANCOVA was conducted using CW as a covariate. The analysis included four groups: unparasitized males, unparasitized females, parasitized males, and parasitized females.

To test the hypothesis that reduced Ave CPL due to sacculinid infection increases crab vulnerability, structural equation modeling (SEM) was performed separately for each sex. SEMs included CW, Ave CPL, and crab fate as observed variables. To improve model fit, paths from CW and infection status to crab fate were excluded. Model fit was assessed using the goodness-of-fit index (GFI), comparative fit index (CFI), and chi-square ( $\chi^2$ ) test. SEMs were constructed using the "lavaan" package (Rosseel, 2012) in R version 4.3.0 (R Core Team, 2023), with the maximum likelihood method used for parameter estimation. Standardized path coefficients (ranging from  $-1$  to  $1$ ) were used to represent the strength and direction of relationships among variables, including both direct and indirect effects on crab loss.

## RESULTS

All sacculinids were identified based on COI sequence similarity (99.63–99.82%) as *Polyascus polygenea* (accession numbers: AY265378).

There was no significant difference in loss rates between unparasitized and parasitized male crabs (Table 1A), nor between unparasitized and parasitized female crabs (Table 1B). Additionally, no significant difference in loss rates was found between males and females within each CW category (Table 2). However, in male crabs, Ave CPL was found to be associated with crab intactness (Table 2). This trend was not observed in females (Table 2).

**Table 1.** Fate of male and female *Hemigrapsus sanguineus* regarding parasitic status by *Polyascus polygenea* in tethering experiments (generalized linear model)

| Host crab type | Intact | Lost | $\chi^2$ | <i>d.f.</i> | <i>p</i> -value |
|----------------|--------|------|----------|-------------|-----------------|
| A. Male        |        |      |          |             |                 |
| Unparasitized  | 15     | 7    | 0.04     | 1           | 0.833           |
| Parasitized    | 15     | 8    |          |             |                 |
| B. Female      |        |      |          |             |                 |
| Unparasitized  | 15     | 8    | 1.75     | 1           | 0.186           |
| Parasitized    | 9      | 11   |          |             |                 |

**Table 2.** Fate of male and female *Hemigrapsus sanguineus* regarding carapace width (CW) and the average propodus length of right and left chelipeds (Ave CPL) in tethering experiments (generalized linear model)

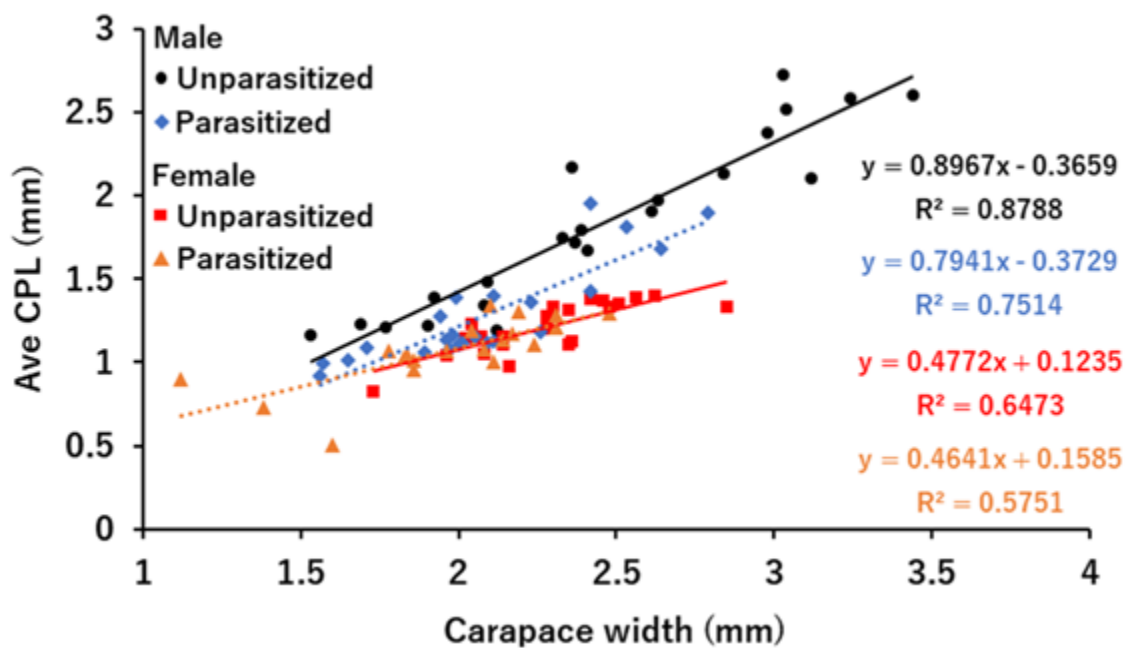
| The body parts compared | Intact | Lost | $\chi^2$ | d.f. | p-value |
|-------------------------|--------|------|----------|------|---------|
| Male                    |        |      |          |      |         |
| CW                      | 30     | 15   | 3.66     | 1    | 0.056   |
| Ave CPL                 |        |      | 4.73     | 1    | 0.030   |
| Female                  |        |      |          |      |         |
| CW                      | 24     | 19   | 1.37     | 1    | 0.242   |
| Ave CPL                 |        |      | 3.34     | 1    | 0.069   |

A significant difference in the slope of the regression of Ave CPL on CW was found between unparasitized males and females (Table 3; Fig. 2). In males infected by *P. polygenea*, Ave CPL was significantly lower than in unparasitized males, and the slope of the Ave CPL–CW regression was

more similar to that of females (Table 3; Fig. 2). Additionally, the slope differed significantly between parasitized males and unparasitized females (Table 3; Fig. 2). Among female crabs, no significant differences in Ave CPL were found between parasitized and unparasitized individuals, as both the slope and intercept of the Ave CPL–CW regression overlapped (Table 3; Fig. 2).

**Table 3.** Results on the effect of carapace width on the average cheliped propodus length (Ave CPL) of *Hemigrapsus sanguineus* concerning sex and parasitic status

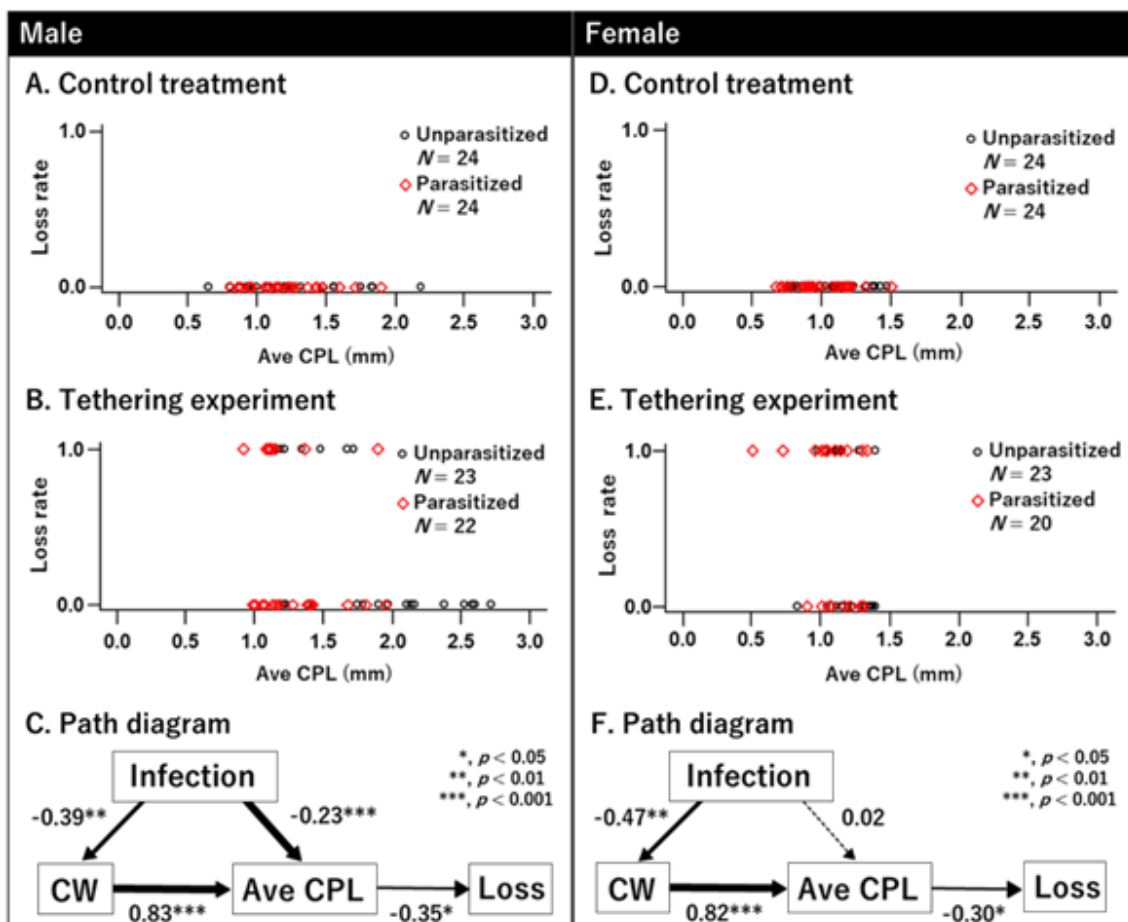
| Comparison of the regression lines          | Homogeneity of the slopes<br>of the regression lines |         | Intercept<br>in Ave CPL |         |
|---------------------------------------------|------------------------------------------------------|---------|-------------------------|---------|
|                                             | F value                                              | p-value | F value                 | p-value |
| Unparasitized male vs. Unparasitized female | 9.66                                                 | 0.003   |                         |         |
| Unparasitized male vs. Parasitized male     | 0.62                                                 | 0.435   | 18.23                   | < 0.001 |
| Unparasitized female vs. Parasitized male   | 5.64                                                 | 0.022   |                         |         |
| Unparasitized female vs. Parasitized female | 0.02                                                 | 0.897   | 0.03                    | 0.860   |



**Fig. 2.** Effect of carapace width on the average cheliped propodus length (Ave CPL) of *Hemigrapsus sanguineus* regarding sex and parasitic status.

SEM showed a good fit to the observed data for both sexes (males: GFI = 0.961, CFI = 0.989,  $\chi^2/df = 1.63$ ,  $p = 0.195$ ; females: GFI = 0.980, CFI = 1.000,  $\chi^2/df = 0.703$ ,  $p = 0.495$ ). No crab loss occurred in the control treatment for males (Fig. 3A). In contrast, in the tethering experiment, male

crabs with an Ave CPL of 1.90 mm or less experienced loss, regardless of infection status (Fig. 3B). The SEM for males revealed that *Polyascus* infection was negatively associated with CW and Ave CPL, and CW was positively related to Ave CPL (Fig. 3C). Furthermore, Ave CPL was negatively associated with crab loss (Fig. 3C). Similarly, no crab loss was observed in the female control group (Fig. 3D). In the tethering experiment, however, crab loss occurred among individuals with an Ave CPL of 1.5 mm or less, regardless of parasitic infection (Fig. 3E). The SEM for females indicated that infection by *P. polygenea* negatively affected CW, and CW was positively related to Ave CPL (Fig. 3F). Additionally, Ave CPL was negatively related to crab loss (Fig. 3F).



**Fig. 3.** Fate of unparasitized (black circles) and parasitized (red diamonds) male crabs in the control treatment (A) and tethering experiment (B), plotted against average cheliped propodus length (Ave CPL). A value of 1.0 on the Y-axis indicates that the crab was lost (presumably due to predation), while 0.0 indicates that it remained intact until the end of the experiment. (C) Path diagram from structural equation modeling showing the relationships among *Polyascus polygenea* infection, carapace width (CW), Ave CPL, and crab fate (lost or intact). (D), (E), and (F) present the same information for female crabs.

## DISCUSSION

In the field survey, no crab loss was observed in the control treatment, where individuals were enclosed in cages, while loss occurred in the tethering experiment. This indicates that the observed losses were likely due to predation. However, our results provide no evidence that infection by the rhizocephalan parasite *P. polygenea* directly increases predation risk in host crabs. In contrast, previous research has shown that the parasitized flatback mud crab *Eurypanopeus depressus* is more susceptible to predation when infected with the rhizocephalan *Loxothylacus panopaei* (Gehman and Byers 2017). Gehman and Byers (2017) proposed that behavioral alterations in parasitized crabs, such as slower escape responses, increase their likelihood of being preyed upon, possibly by making them easier or more attractive targets for predators. Furthermore, rhizocephalan infections are known to alter host grooming and burying behaviors, with these behaviors varying according to the maturity of the externa (Bishop and Cannon 1979; Innocenti et al. 1998; Vázquez-López et al. 2006). In the swimming crab *Callinectes rathbunae* parasitized by *Loxothylacus texanus*, *C. rathbunae* carrying mature externae did not exhibit burying behavior, possibly because they devoted more time to grooming and ventilating the externa, and showed increased feeding activity compared to individuals with immature externae (Vázquez-López et al. 2006). The same study also reported that crabs with three to four mature externae on their abdomens exhibited lower levels of aggression than those with only one or two mature externae, or with immature externae. In the present study, only parasitized crabs with mature externae were used. However, we did not record the number of externae per individual. It is possible that these parasitized crabs were less aggressive and less inclined to hide than unparasitized ones, potentially increasing their vulnerability to predation. Nevertheless, because the tethering experiment restricted the ability to hide for both parasitized and unparasitized individuals, this limitation may have affected the observed predation rates. Further research is needed to examine potential behavioral changes in infected hosts. For instance, comparing the escape speed and burying behavior of parasitized and unparasitized individuals, grouped by the number of externae, may help reveal subtle behavioral effects of infection that were not detected in the present study.

The ANCOVA result indicated that *Polyascus* infection suppressed both CW and Ave CPL in male crabs, consistent with morphological feminization previously reported in this and other studies (Hartnoll 1962; Yamaguchi and Aratake 1997; Kristensen et al. 2012; Toyota et al. 2023). The reduction in male cheliped size in this study was evaluated using Ave CPL as an indicator of morphological feminization, focusing on its role in defense against predators. This approach differs from previous studies that examined only the phenomenon of feminization caused by rhizocephalans, using other metrics such as CPL (Hartnoll 1962; Toyota et al. 2023), chela height

(Yamaguchi and Aratake 1997), and claw height (Kristensen et al. 2012).

SEM results suggest that the parasite may directly inhibit the growth of Ave CPL through a physiological mechanism. Alternatively, given the strong positive correlation between CW and Ave CPL, the parasite may indirectly reduce Ave CPL by suppressing CW growth. Moreover, SEM showed that a one-unit increase in Ave CPL was associated with a 0.35 decrease in the probability of crab loss. This indicates that *Polyascus*-induced reductions in Ave CPL increase vulnerability to predation in males. In female crabs, ANCOVA detected no significant reduction in Ave CPL due to infection. However, SEM revealed a negative effect of *Polyascus* infection on CW and a positive relationship between CW and Ave CPL. No direct effect of infection on Ave CPL was observed in females, possibly due to their inherently smaller chelipeds. Nevertheless, SEM results showed that a one-unit increase in Ave CPL reduced the probability of loss by 0.30. This suggests that the infection-induced reduction in CW led to decreased Ave CPL, indirectly increasing predation risk. Overall, these findings demonstrate that *Polyascus* infection increases host susceptibility to predation through indirect morphological changes—non-consumptive effects in the sense of Peacor et al. (2020). This interpretation aligns with the GLM analysis of Ave CPL, which demonstrated a significant association between Ave CPL and loss of male hosts. Thus, the reduction in CPL caused by rhizocephalan infection may play a particularly important role in increasing predation risk in male crabs.

Several studies have suggested that rhizocephalan parasites may influence predation risk in their hosts through behavioral modifications. For example, in *E. depressus* parasitized by *L. panopaei* and in the blue swimming crab *Portunus pelagicus* parasitized by *Sacculina granifera*, infection inhibits burying behavior and increases the time spent on land for grooming of the externa (Bishop and Cannon 1979; Gehman and Byers 2017). Additionally, *E. depressus* parasitized by *L. panopaei* exhibit reduced feeding behavior and activity levels compared to unparasitized individuals, suggesting that parasitized crabs spend more time hiding than foraging (Belgrad and Griffen 2015). In *P. pelagicus* parasitized by *S. granifera* and in the portunid crab *Charybdis longicollis* parasitized by *Heterosaccus dollfusi*, individuals exhibit lower levels of aggression compared to unparasitized conspecifics (Innocenti et al. 1998, 2003). This reduced aggression is considered to help avoid injury and thereby prolong the survival of both the host and the parasite (Innocenti et al. 2003). In contrast, relatively few studies have examined whether rhizocephalan-induced morphological changes contribute to increased predation risk, although such a possibility has been proposed (Gehman and Byers 2017; Galil and Innocenti 2024). The present study provides the first empirical evidence that morphological changes caused by rhizocephalan infection—specifically, reductions in cheliped size—can weaken the defensive capabilities of the host and thereby increase its vulnerability to predation. However, we did not directly observe predator

species or the defensive behavior of host crabs in response to predatory threats. Two species of Japanese pufferfish (*Takifugu poecilonotus* and *Takifugu niphobles*) and the scorpionfish *Sebastiscus marmoratus* have been reported as predators of crabs (Thanh et al. 2003). Around the field site of the present study, both *T. niphobles* and *S. marmoratus* are reportedly caught by local fishers (personal communications), suggesting they are likely predators of *H. sanguineus*. Octopuses are also occasionally caught in the same area, although their exact species remains unidentified. In addition, herons (family Ardeidae) were observed during the experiment; while the species was not identified, they may also act as potential predators. To fully assess the ecological impact of rhizocephalan infection on crab populations, it will be essential to identify the main predators in the study area and investigate their interactions with parasitized hosts.

Rhizocephalans play an important role in marine food webs by indirectly influencing the dynamics of species associated with their hosts, such as predators and prey, through alterations in host behavior, physiology, and morphology (Mouritsen and Poulin 2002; Toscano et al. 2014; Gehman and Byers 2017). For example, the crab *E. depressus* parasitized by *L. panopaei* has been shown to reduce its consumption of the mussel *Brachidontes exustus*, a primary prey item (O'Shaughnessy et al. 2014; Toscano et al. 2014). Toscano et al. (2014) proposed that the parasite's invasion of internal organs impairs digestive function, prolongs digestion time, and ultimately decreases both foraging effort and prey intake. These feeding reductions in infected crabs may, in turn, benefit conspecifics by increasing mussel abundance, which creates a predation refuge and promotes the growth of unparasitized crab populations. Similarly, Belgrad and Griffen (2015) suggested that parasitized *E. depressus* individuals spend more time hiding than foraging, which may reduce their predation risk and, in turn, influence community structure and trophic interactions. Additionally, Gehman and Byers (2017) reported that the predatory crab *Callinectes sapidus* preferentially preys on *E. depressus* individuals infected by *L. panopaei*. According to Gehman et al. (2017), the frequency of predation is positively correlated with the prevalence of parasitized hosts, possibly due to reduced host mobility caused by infection. In this study, we demonstrated that rhizocephalan infection increases host susceptibility to predation through parasite-induced morphological changes. However, to fully understand the ecological implications of rhizocephalan parasitism, long-term monitoring of predator, prey, and parasite populations at the study site will be essential. Such data would provide a clearer understanding of the complex, cascading interactions mediated by rhizocephalans in marine ecosystems.

## CONCLUSIONS

Rhizocephalans are key parasites of decapod crustaceans, known for inducing parasitic castration, suppressing the development of secondary sexual characteristics, feminizing male hosts, and altering host behavior. This study is the first to provide empirical evidence that infection by the rhizocephalan *Polyascus polygenea* increases the vulnerability of the Asian shore crab (*Hemigrapsus sanguineus*) to predation by reducing chela size, a critical defensive trait. Although the parasite did not directly increase the likelihood of crab loss in either sex, parasitized males exhibited significantly shorter chelipeds compared to unparasitized males, and smaller cheliped size was associated with a higher risk of predation. In females, *P. polygenea* infection negatively affected average cheliped propodus length indirectly through its suppression of carapace width growth, which also contributed to increased predation risk. These findings demonstrate that rhizocephalan parasites can increase host susceptibility to predators through indirect morphological changes, constituting a non-consumptive effect. This study offers new insight into the broader ecological impacts of rhizocephalan infection and highlights their potential role in shaping marine community dynamics through host modification.

#### List of abbreviations

ANCOVA, analysis of covariance.

Ave CPL, the average value of right and left cheliped propodus length.

CFI, comparative fit index.

CPL, cheliped propodus length.

CW, carapace width.

GFI, goodness-of-fit index.

SEM, structural equation modeling.

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**Authors' contributions:** AK and YY designed the research. AK and KT conducted the sampling. AK performed the morphological measurements, field survey, data analysis, and wrote the first draft of

the manuscript. KT carried out DNA sequencing. AK and KT analyzed the morphological data. KT and YY improved the manuscript.

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