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## Infestation and effect of parasitic isopod *Epipenaeon ingens ingens* Nobili, 1906 on commercial shrimp species in the eastern Mediterranean: a case study of the population of brown shrimp *Penaeus aztecus* Ives, 1891

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**Abstract:** Monthly samples of seven-species of penaeid shrimps, namely, *Penaeus aztecus* (Ives, 1891), *Penaeus hathor* (Burkenroad, 1959), *Penaeus japonicus* (Spence Bate, 1888), *Penaeus kerathurus* (Forskål, 1775), *Penaeus semisulcatus* (De Haan, 1844), *Metapenaeus monoceros* (Fabricius, 1798) and *Metapenaeopsis aegyptia* (Galil & Golani, 1990) were collected from Antalya Bay in the Eastern Mediterranean Sea from March, 2019 through March, 2020. Except for *P. aztecus* and *P. semisulcatus*, no other shrimp species were infected with the parasitic isopod *Epipenaeon ingens ingens*. Of the 3043 *P. aztecus* shrimp inspected, 541 individuals were infected (17.8%) with the bopyrid parasite *E. ingens ingens*. However, the annual infestation rate in recruiting year class (28 mm < CL: 5.7%) was much less than in larger and sexually mature (CL ≥ 34 mm: 36.2%) individuals. Monthly infestation rates varied from 0.86 to 84.8%, and the size of infested shrimps ranged from 20 mm to 44 mm CL. The left branchial chamber was more likely infected (43.1%) than the right one (39.8%), and 17.1% appeared to be doubly infected. The percentage of the ovigerous female *E. ingens ingens* fluctuated between 31.7% (August) and 90.0% (February). We report for the first time the size at first accompaniment (FAS<sub>50%</sub>) and at first maturity (FMS<sub>50%</sub>) for the female parasite *E. ingens ingens*, 11.0 mm TL and 13.0 mm TL, respectively. The gonad weight (0.779 ± 0.73 g) and GSI (0.223 ± 0.333) value of uninfected female *P. aztecus* were about eight times greater than it was for infected shrimps (0.094 ± 0.18 g and 2.006 ± 1.669, respectively).

**Key words:** Bopyrid isopod, *Epipenaeon ingens*, brown shrimp, *Penaeus aztecus*, *Penaeus semisulcatus*, parasite

### 1. Introduction

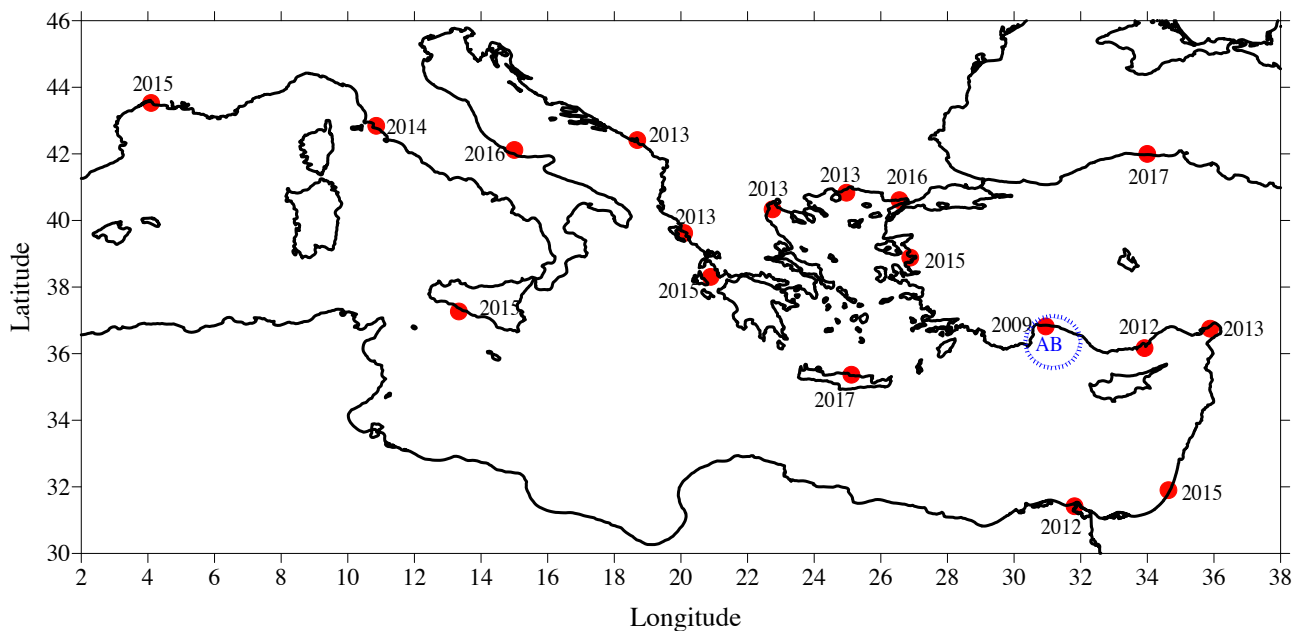
The Northern brown shrimp *Penaeus aztecus* Ives 1891, the most commercially important and native to Western Atlantic, was first recorded in the Mediterranean Sea in December 2009, and it was hypothesized that its introduction to the eastern Mediterranean Sea was due to ballast water (Deval et al., 2010). Within only eight years after the first finding, its distribution area has expanded very quickly in the northern areas (Egypt, Israel, Turkey, Greece, Montenegro, Italy, Malta and France) of the line between Egypt and France (Figure 1).

The female bopyrid isopods release epicaridium larvae that parasitize calanoid copepods, which are their intermediate hosts. The epicaridium larva metamorphoses into a microniscus larva and then a cryptoniscus larva that settles onto a definitive crustacean host (Williams and Boyko, 2012). The sex of parasites is under epigenetic control wherein the first undifferentiated juvenile (bopyridium) to settle becomes a female (Reinhard, 1949; Reverberi, 1947). Subsequent cryptoniscus larvae that

enter the branchial chamber of the infected host will attach to the female parasite and will become male (Anderson, 1975; Dale and Anderson, 1982; O'Brien and Van Wyk, 1985) within 24 h after contact with the females (Anderson, 1990). Parasitism poses a metabolic drain on the decapod host because females feed on haemolymph and ovarian fluids after piercing the inner cuticle of the host (Burse, 1978). Effects on the host are manifold (lower fecundity, castration, reduced moult frequency, etc.) and can be dramatic (Klompaker et al., 2018).

The parasitic *Epipenaeon ingens ingens* Nobili, 1906 was one of the most common bopyrids, whose complete life cycle follows other bopyrid isopods (Smith et al., 2008) and who was first described by Nobili (1906) on the green tiger shrimp *Penaeus semisulcatus* De Haan, 1844 from the Red Sea. *Epipenaeon ingens ingens* is mainly known from the Indian Ocean (Devi, 1982; Ravichandran et al., 2000; Thirumilu, 2011) and the western Pacific Ocean (Owens and Rothlisberg, 1995; An et al., 2014), and it has been reported also in various nearby regions such as Suez

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**Figure 1.** The finding years and locations for *P. aztecus* in the Mediterranean and Black Seas (modified from Gönülal and Türetken, 2019) (Blue circle indicates the study area, AB: Antalya Bay).

Canal (Nierstrasz et al., 1931) and Eastern Mediterranean (Bourdon, 1968). In all spreading areas, 13 different Penaeid shrimps have been infected by *E. ingens ingens* (An et al., 2014), and the two most heavily parasitized species are *P. semisulcatus* and *Penaeus japonicus* Spence Bate, 1888 (Sivasubramanian et al., 2016). The coexistence between the definitive host *P. aztecus* and her parasitic guest *E. ingens ingens* was reported three years after (Korun et al., 2013; Teker, 2016) the first finding of *P. aztecus* in the Mediterranean. No information is yet available on the coexistence in any of the other 17 studies (Özcan et al., 2019) showing the distribution locations of *P. aztecus* records in the Mediterranean (Figure 1).

In the first of the two studies that were concluded before the beginning of our study, only some biometric characteristics of *E. ingens ingens* from infected *P. aztecus* (Korun et al., 2013), and in another study where some reproductive characteristics of *P. aztecus* were examined (Teker, 2016), only mean infestation rates were presented. Our study aimed at obtaining more detailed monthly information (prevalence, reproduction) about the infestation of the parasitic guest *E. ingens ingens* from commercial shrimp species in Antalya Bay, since these can affect the morphology, fertility, and weight of host shrimp *P. aztecus*.

## 2. Material and methods

### 2.1. Study area and sampling

Sampling was carried out during the night time, on board R/V "Akdeniz Su" using a standard otter-trawl net with

monthly periodicity from March 2019 to February 2020 in the Antalya Bay, Eastern Mediterranean. The sampling area is about 4 km from the shore and has a depth of 25–50 m (Figure 1). Individuals of all shrimp species were immediately frozen by seawater in the cold storage on board and brought back to the laboratory for subsequent biological studies. According to sea surface temperature (SST) data obtained from the Turkish General Directorate of Meteorology, the monthly SST value fluctuated between 16.0 °C in February and 29.4 °C in August, with an annual mean of  $22.7 \pm 4.7$  °C.

### 2.2. Data collection

Firstly, each species and sexes were carefully separated. Then carapace length (CL, 0.1 mm) was measured with an electronic calliper for all specimens ( $n = 3043$ ). To evaluate the recruitment of *P. aztecus*, the number of recruits in each month was calculated by the analysis of the mean CL of the first modal groups ( $CL < 28$  mm) and termed as young-of-the-year. The body weight (TW, 0.01 g) with digital balance was weighed for 833 specimens. The parasitic bopyrid species *E. ingens ingens* was identified according to the description of Bourdon (1968) and An et al. (2014).

Of 541 infected host shrimps *P. aztecus*, the presence of a bopyrid on the right or left branchial chambers on the 380 hosts was noted. Four different types of host shrimp were classified: *i*) apparently uninfected female (UI-F), *ii*) male (UI-M), *iii*) infected female (In-F), and *iv*) male (In-M). Prevalence was estimated as the proportion of infected shrimps in a given time period (month or year). All parasites were removed from their hosts to measure their

sizes and to appreciate their status and were photographed with a scale. Digital images were analysed using Image J software to measure the sizes of *E. ingens ingens* specimens after calibration with scale micrometers. The total length (TL: from the anterior margin of the cephalon to the posteriormost edge of the pleotelson) and maximum width (width of pereomeres 4) of the female and male parasites were measured. The sex of *E. ingens ingens* was identified according to the description of Bourdon (1968) and An et al. (2014).

Female parasites without eggs or embryos in their brood pouch were classified as sexually immature (Im-F), the rest of them as ovigerous (Ov-F) females. Eggs and larvae from the marsupium of two females of *E. ingens ingens* were removed (one by one) and suspended in 70% alcohol. The number of eggs in five 50- $\mu$ L samples taken using a micropipette (Owens, 1983) was placed in a 3 cm<sup>2</sup> circular cavity slide, digitally photographed with an Olympus DP11 microscope camera and counted, and the mean count was used in the final estimation of total egg number and epicardium larvae.

To determine the effect of the parasite on the host shrimps; i) after measuring the bodyweight of both infected- and uninfected (a total of 343) female shrimps (CL  $\geq$  33 mm), gonads were taken carefully and their weights (GW, 0.001 g) were measured. Variation in reproductive activity was assessed by the gonadosomatic index (GSI) value, which was estimated as  $GSI = (GW \div TW) \times 100$ , ii) the patesma with first pair pleopods from 54 male shrimps (CL  $\geq$  28 mm), both infected- and uninfected specimens were taken, and their lengths were measured using ImageJ software.

### 2.3. Statistical analysis

Descriptive statistics were reported as means  $\pm$  standard deviations (sd) and univariate techniques (One-way ANOVA, Chi-square, Mann–Witney, Kruskal–Wallis tests), were performed to compare means using SPSS version 17.0 (SPSS, 2008). A comparison of regressions lines was carried out using the Statistical tool (command of 'actool') of MATLAB version 7.0 (MathWorks) ANOCOVA. Size at first accompaniment (FAS<sub>50%</sub>; the TL at which 50% of the female parasites have a male parasite) and at first maturity size (FMS<sub>50%</sub>; TL at which 50% of the female parasites have reached maturity) of the female parasites were modelled using the logistic curve:

$$r(l) = \left[ \frac{\exp(v_1 + (v_2 \times l))}{1 + \exp(v_1 + (v_2 \times l))} \right]$$

using the software CC2000 (ConStat, 1995), where  $r(l)$  is the probability for each 1-mm size class ( $l$ ), and  $v_1$  and  $v_2$  are regression parameters to be estimated.

## 3. Results

### 3.1. Size distribution of infected shrimps and prevalence of *E. ingens ingens*

Of the 3043 *P. aztecus* shrimp inspected, 541 individuals were infected (17.8%) with the bopyrid parasite *E. ingens ingens* (Table 1). During the study period, only one female *P. semisulcatus* out of 124 specimens examined was found with *E. ingens ingens* in March 2019. No other penaeid shrimp species known to be in the study, such as *P. japonicus* (473 indiv.), *Metapenaeus monoceros* (Fabricius, 1798) (184 indiv.), *Penaus kerathurus* (Forskål, 1775) (15 indiv.), *Metapenaeopsis aegyptia* (Galil & Golani, 1990) (65 indiv.) and *Penaus hathor* (Burkenroad, 1959) (18 indiv.), were infected with bopyrids.

The size of *P. aztecus* ranged from 16.4 mm to 47.3 mm CL, with a significantly smaller mean size of  $26.7 \pm 3.6$  mm for males versus  $31.7 \pm 4.9$  mm for females (ANOVA,  $F = 257.935$ ;  $p = 0.000$ ). The monthly percentage of infestation changed significantly ( $X^2 = 44.013$ ;  $p = 0.000$ ) between 0.86% (July) and 84.8% (March). The infection rates of male and female shrimps did not differ significantly (16.4% and 19.4%, respectively;  $\chi^2 = 0.019$ ,  $df = 1$ ,  $p > 0.05$ ). The infestation was highly positively related to size of host ( $r = 0.925$ ,  $p = 0.000$ ). The mean CL values of the In-M ( $30.7 \pm 3.8$  mm) and In-F ( $34.2 \pm 4.5$  mm) shrimps were larger than for the UI-M ( $25.8 \pm 2.9$ ) (ANOVA,  $F = 107.444$ ,  $p = 0.000$ ) and UI-F ( $31.1 \pm 4.7$ ) (ANOVA,  $F = 27.210$ ,  $p = 0.000$ ), respectively. No specimens of *P. aztecus* were parasitized with smaller than 20 mm CL for both sexes. Although 50% of individuals were infected in all length classes over 30 mm in males, the infection rate reached 50% at the 42 mm length class in females (Figure 2).

Monthly 30–40 individuals belonging to the 2018 cohort with a high infection (52–92%) were sampled during spring 2019. The recruits started to come to the fishing area intensively in July 2019 and were almost parasite-free (only 0.3% in July), causing the prevalence of the infection in the population to decrease to 0.86%. The recruited year class constitutes more than half of the shrimp population between July and September 2019, when the SST value was at maximum. Then, the parasite *E. ingens ingens* attaches to the new recruits during their growth in the recruitment area (therewithal fishing area), and the prevalence reached 25%–30% in the recruiting year class during autumn 2019. However, there was already a high prevalence (~50%) in large individuals (2018 cohort: CL  $\geq$  34 mm) at the beginning of autumn, reaching 100% at the end of winter (February 2020) (Table 1).

Due to the high positive correlation ( $r = 0.749$ ,  $p = 0.005$ ) between SST and the percentage of newly recruited year class, the massive recruitment of young and parasite-free shrimps in the population occurs between July–September when the water temperature is at maximum

**Table 1.** Monthly average sea surface temperatures (SST) between March 2019 and February 2020 and percentage of infected *P. aztecus* sampled from Antalya Bay (M: male, F: female, CL: carapace length).

Month	SST	Mean	F + M		M		F		CL < 28 mm		CL ≥ 34	
		CL±sd	n	%inf	n	%inf	n	%inf	n	%inf		
Mar	16.2	33.9 ±8.6	46	84.8	22	77.3	24	91.7	-	-	26	92.3
Apr	18.1	35.9 ±9.7	34	61.8	17	64.7	17	58.8	-	-	25	72.0
May	20.3	36.3 ±9.9	30	46.7	16	62.5	14	28.6	-	-	23	52.2
Jun	23.8	31.1 ±9.6	44	11.4	28	0.0	16	31.3	7	0.0	14	35.7
Jul	27.3	<b>26.5 ±2.1</b>	<b>1286</b>	<b>0.86</b>	749	0.4	537	1.5	835	0.5	61	13.1
Aug	29.4	30.9 ±2.3	794	15.2	370	17.8	424	13.0	179	8.8	278	7.9
Sep	28.4	27.3 ±4.3	296	27.4	154	23.4	142	31.7	158	23.6	34	47.1
Oct	25.4	30.2 ±5.6	214	37.9	99	41.4	115	34.8	26	26.9	62	48.4
Nov	22.8	32.1 ±4.9	156	55.1	84	58.3	72	51.4	23	26.1	70	67.1
Dec	20.8	33.6 ±8.6	47	-	13	-	34	47.0	5	0.0	25	60.0
Jan	17.8	32.9 ±7.8	60	66.7	30	50.0	30	83.3	5	0.0	27	88.9
Feb	16.0	34.2 ±9.7	36	72.2	16	75.0	20	70.0	2	0.0	20	100.0
			3043	17.8	1598	16.4	1445	19.4	991	5.7	665	36.2

(27.3–29.4 °C), and, thus, the proportion of parasitized individuals in the population suddenly drops (Table 1).

### 3.2. Parasite

Descriptive statistics of parasites are presented in Table 2. The size of female *E. ingens ingens* ranged from 2.5 mm to 26.8 mm in TL and from 1.0 mm to 20.9 mm in width. TL and width of males varied from 2.0 mm to 8.7 mm and 1.0 mm to 3.7 mm, respectively. The mean TL of females (12.2 ± 5.8 mm) was 144% larger than males (5.0 ± 1.2 mm). There were no statistical differences between TL versus attached side of host for both male (F = 1.710, p = 0.184) and female (F = 2.470, p = 0.086) bopyrid parasites. Regression analysis showed significant positive relationships between host shrimp size (CL) and TL of female parasites, expressed as follows (r = 0.604; F = 99.02, p = 0.000).

$$TL (mm) = -8.435 + 0.687 \times CL (mm)$$

The minimum size of the recently settled juvenile (bopyridium, <5 mm TL) was 2.5 mm, and 65% of them were found in October. When the bopyridium reaches 5 mm in TL, a male begins to accompany it (18.2%), and this ratio reaches 80% at 13 mm TL (Figure 3). There were 47.3% of the female parasites accompanied by a male on their pleon, and 40.6% of females were found with eggs in their brood pouch. In ten female parasites, the males were absent (other parasites had apparently died or had been lost during collection), but eggs were present. The smallest bopyrid to have eggs in its marsupium was 7.2 mm TL. The largest virgin female, as indicated by lack of eggs, was 10

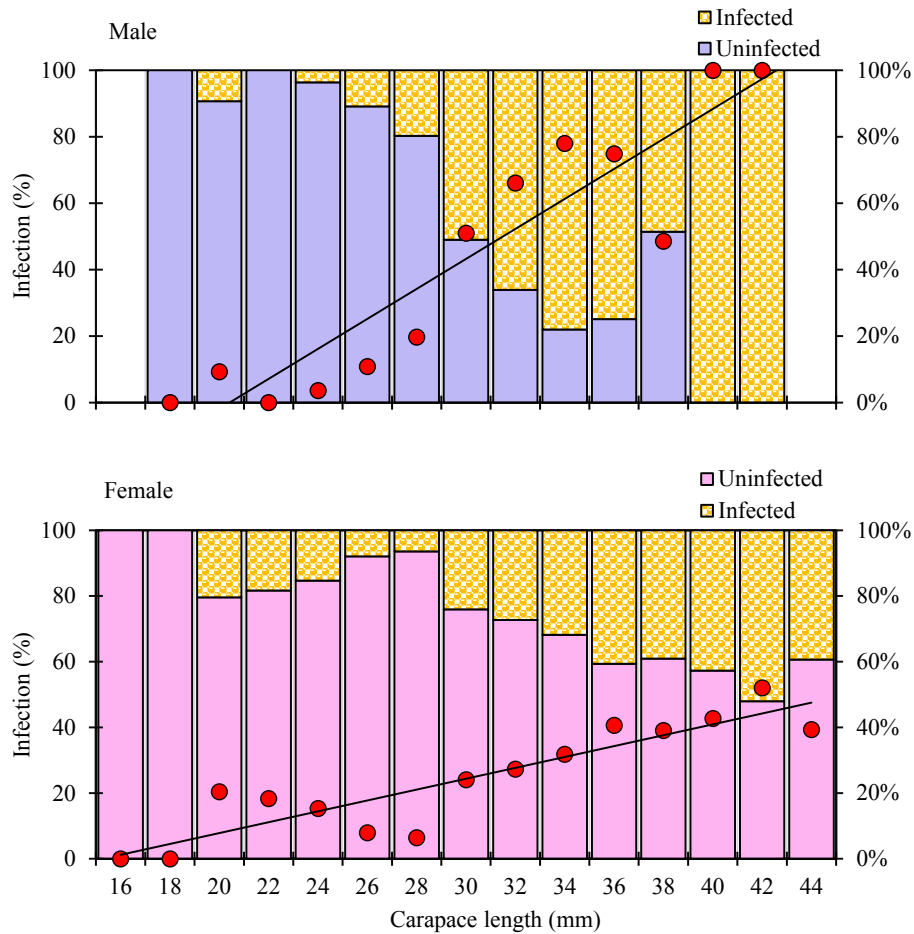
mm. The TL of the Ov-F parasite ranged from 7.2 to 26.8 mm, with a mean of 17.4 ± 4.2 mm.

While the presence of Ov-F bopyrids was encountered throughout the year, the percentage fluctuated between 31.7% (August) and 90.0% (February). The season was not a significant factor in the presence of Ov-F (F = 1.088, p = 0.408). There was a statistically insignificant correlation between Ov-F and SST (r = -0.503, p = 0.095). The fitted logistic curves revealed at first accompanied (FAS<sub>50%</sub>) and first maturity (FMS<sub>50%</sub>) sizes of 11.0 mm TL and 13.0 mm TL, respectively, for female parasites (Figure 4).

### 3.3. Effects on host

The branchial chamber of parasitized *P. aztecus* developed a characteristic bulge. The left branchial chamber being higher infected (43.8%) than the right one (38.2%), and 18% appeared to be doubly infected. Season (p = 0.907), sex (p = 0.482) and size of host shrimp (p = 0.707) have no effect on this differentiation. The number of parasites on the host varied between 1 and 4.

Comparison of regression lines—TW versus CL by infection status for both male (n = 354) and female (n = 433) shrimps—are shown in Figure 5. Analyses revealed a statistically significant difference between the b-values of the TW/CL relationship between infected and uninfected only for male shrimps (F = 3255.9, p = 0.000) (Figure 5a and 5b). Because the p-value was bigger than 0.05, there was not a statistically significant relationship between the variables at the 95.0% confidence level for female shrimps (Figures 5c and 5d).



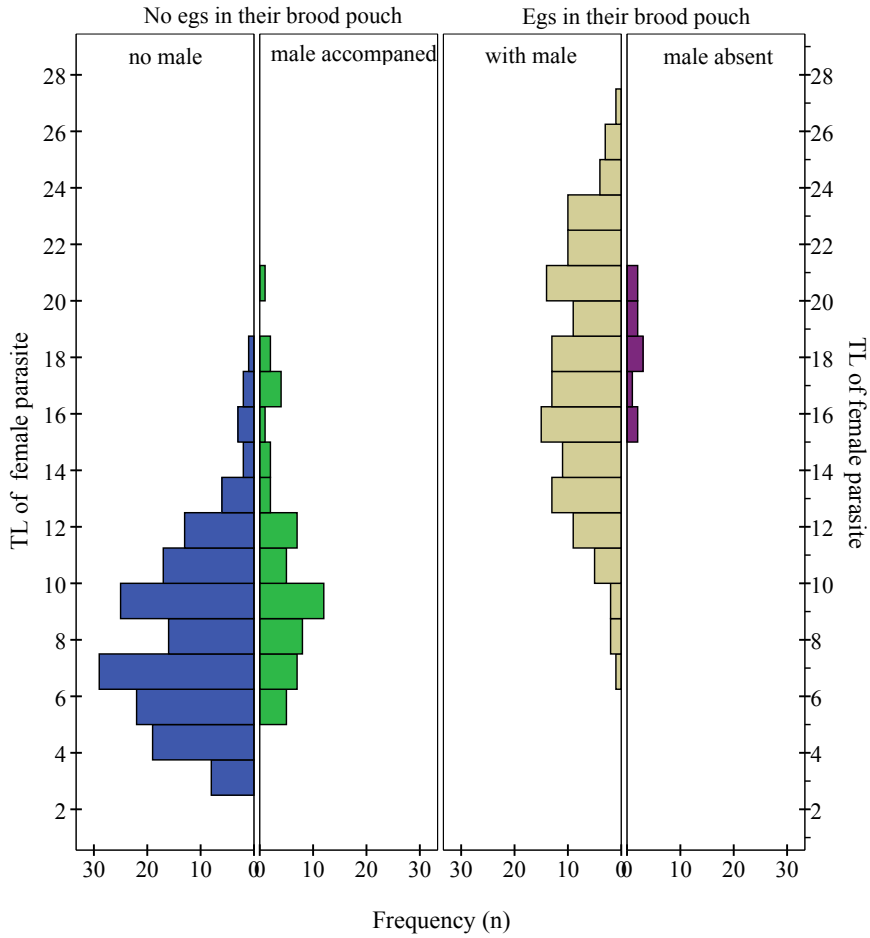
**Figure 2.** Percentage of infected and uninfected shrimps by carapace length (CL) and by sex of *P. aztecus* sampled in Antalya Bay (red circular: an observed percentage of the infection, thick line: correlation line).

**Table 2.** Descriptive statistics of the male and female parasite (M: male. F: female. N: number. TL: total length. W: width).

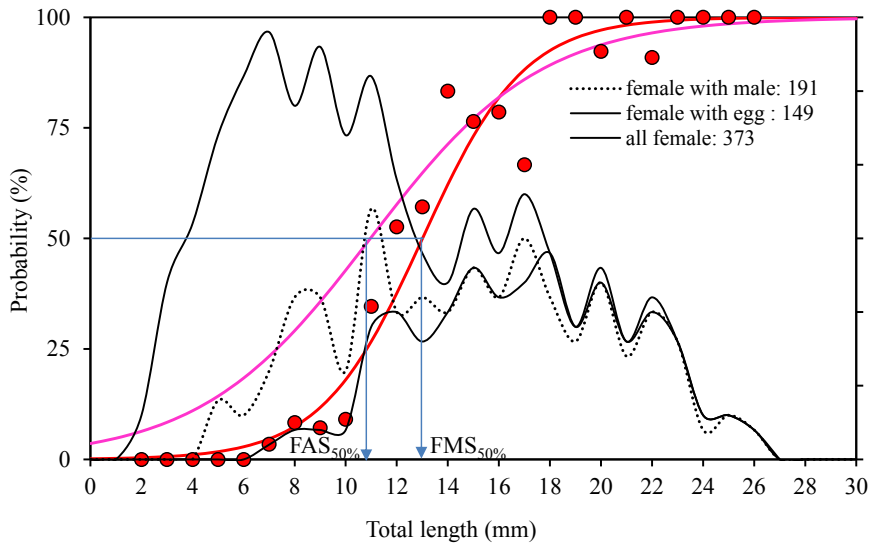
Sex	Status	N	TL (mm)	Range (mm)	W (mm)	Range (mm)	One-Way ANOVA			
							TL	W	TL/W	
F	all	375	12.2 ± 5.8	2.5 - 26.8	9.5 ± 4.9	1.0 - 20.9				
	Ov-F	148	<b>17.4 ± 4.2</b>	7.2 - 26.8	13.0 ± 4.95	5.9 - 20.9	F =	211.06	118.08	21.35
	Im-F	225	<b>8.8 ± 3.7</b>	2.5 - 26.4	6.4 ± 3.5	1.0 - 18.8	p =	0.000	0.000	0.000
M	all	174	5.0 ± 1.2	2.0 - 8.7	1.8 ± 0.50	1.0 - 3.7				
	by Ov-F	124	5.3 ± 1.2	2.0 - 8.7	2.2 ± 0.47	1.1 - 3.7	F =	27.76	18.87	0.730
	by Im-F	50	4.3 ± 1.0	2.8 - 6.6	1.8 ± 0.49	1.0 - 2.9	p =	0.000	0.000	0.397

There was no difference ( $F = 1.952, p = 0.164$ ) in terms of the mean TW between In-F ( $37.3 \pm 3.7$  mm CL) and Un-F ( $37.9 \pm 3.9$  mm CL) shrimps ( $CL \geq 33$  mm) with equal mean lengths (In-F=  $37.3 \pm 3.7$  mm CL and Un-F=  $37.9 \pm 3.9$  mm CL).

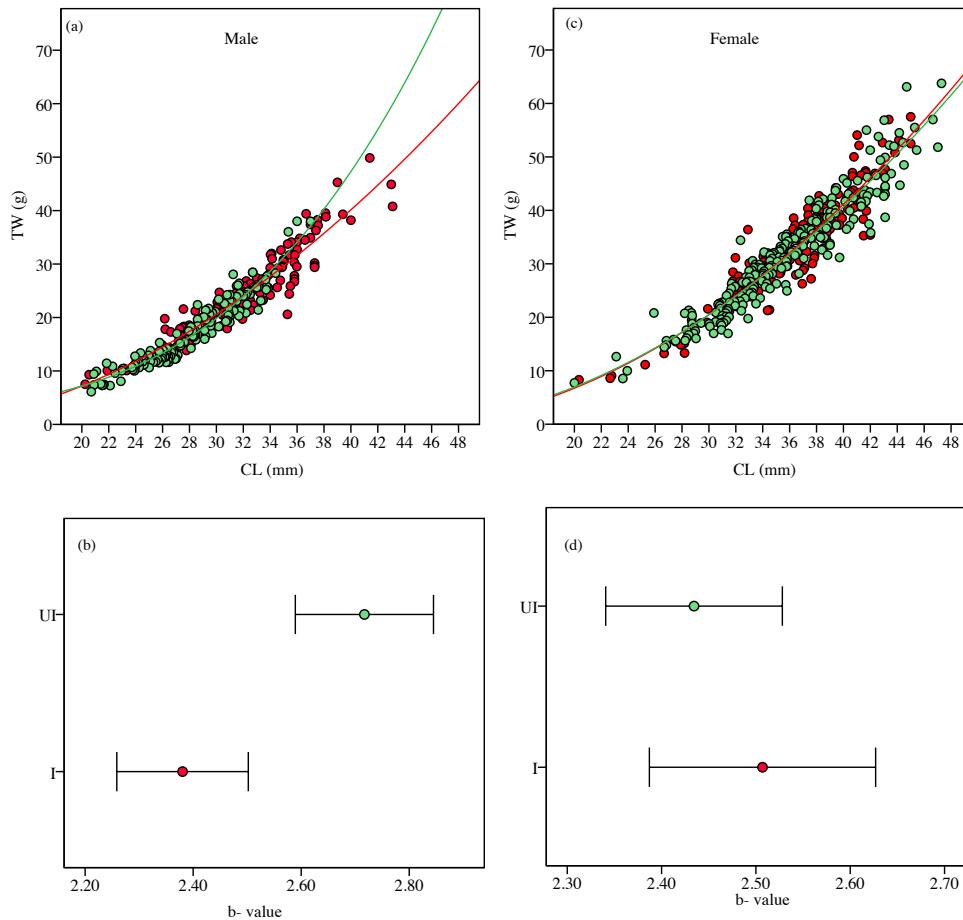
However, ovaries were undeveloped whatever the host size and the season. Both the GW ( $0.667 \pm 0.503$  g) and GSI value ( $1.77 \pm 1.19$ ) of Un-F were about ten times greater than In-F shrimps ( $0.067 \pm 0.083$  g and  $0.18 \pm 0.19$ , respectively) (for GW;  $F = 193.9, p = 0.000$  and for GSI;  $F =$



**Figure 3.** Length frequency distribution of female *E. ingens ingens* according to two different statuses.



**Figure 4.** Data of *E. ingens ingens*. Expected (pink logistic line) percentage of a female with male, expected (red logistic line) and observed percentage (red circular) of Ov-F, and size structure of female parasite (thick line), female accompanied with male (broken line) and Ov-F (thin line), size at first accompaniment ( $FAS_{50\%}$ ) and size at first maturity ( $FMS_{50\%}$ ).



**Figure 5.** Data of *P. aztecus*. The CL/TW relationship of male (a) and female (c) host. Multiple comparisons result to determine which biometric relationships (slope: b value) are significant only for male shrimps. Horizontal lines denote statistical confidence limits for the b-value of both sexes: male (b) and female (d).

236.1,  $p = 0.000$ ) (Figure 6). No significant difference was found between the length of patesma in In-M ( $8.07 \pm 0.87$  mm) and Un-M ( $8.36 \pm 0.63$  mm) ( $F = 193.9$ ,  $p = 0.174$ ).

### 3.4. Eggs and larvae of *E. ingens ingens*

The marsupium of the female parasite was filled with brood or embryos that appeared white/pale or pale yellow, and the eggs were visible to the naked eye (Figure 7a). One mature female *E. ingens ingens* had 83 360 eggs and the other had 65 600 epicaridium larvae. Mean diameter of eggs was  $147.9 \pm 12.3$   $\mu\text{m}$  ( $n = 350$ ). Marsupium of the female parasite was full of the hatched embryo (epicaridium larvae) that appeared as a dark mass (light brown). The average sizes of the larvae, recognised by their free appendages and well-developed eyespots were TL =  $262.1 \pm 12.7$   $\mu\text{m}$  ( $n = 50$ ) and width =  $169.1 \pm 5.0$   $\mu\text{m}$  ( $n = 50$ ) (Figure 7b).

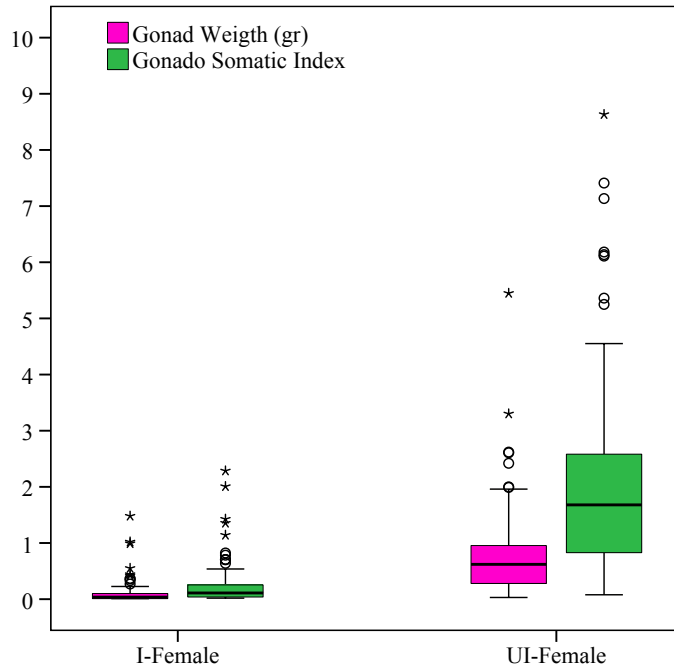
## 4. Discussion

There is limited information about the isopod parasite *E. ingens ingens* and its host species in the Mediterranean.

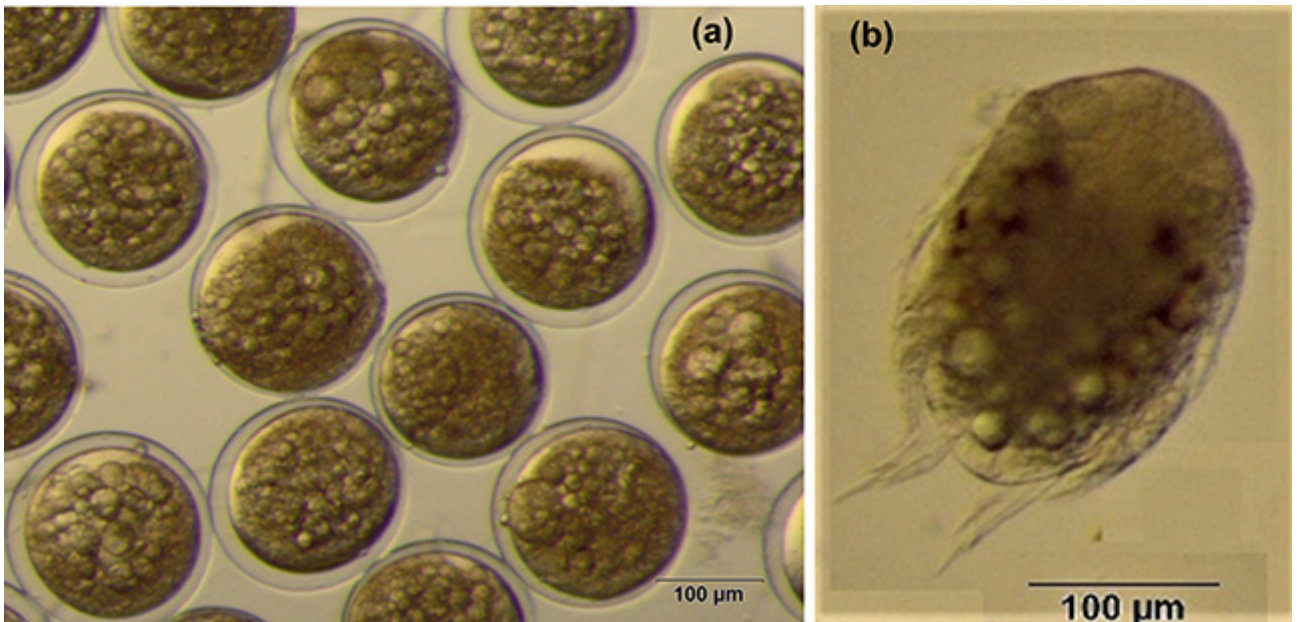
After about 60 years from the first identification in the Red Sea (Nobili, 1906), *E. ingens ingens* was identified in the Gulf of Mersin (eastern Mediterranean coast of Turkey) on the same host species, *P. semisulcatus* (Bourdon, 1968). After two decades, *E. ingens ingens* infested the blue shrimp *Penaeus stylirostris* Stimpson, 1871 in Israel (Overstreet, 1987). Only three years after the first finding of *P. aztecus* in the Mediterranean, *E. ingens ingens* preferred *P. aztecus* as its new host (Korun et al., 2013). While the infestation rate of *P. aztecus* individuals sampled during 2015 was reported as 7.5% in the study of Teker (2016), in another study in 2016, which included the same researcher in the same field, very interestingly, the mean infestation rate was calculated as 59.02% (Gökoğlu et al., 2020). The fact that *P. aztecus*, which was examined by researchers, consists of only large individuals (CL mm 30 mm) explains this high and incorrect infection rate.

*Penaeus aztecus* spawns offshore at depths usually exceeding 18 m during most of the year (Larson et al., 1989). Surface current transports post-larvae towards





**Figure 6.** Box and Whisker plot representation of the negative effect of *E. ingens ingens* on the gonadal development of the female host shrimp *P. aztecus*.



**Figure 7.** Developing embryos (a) and newly hatched epicaridium larvae (b) of *E. ingens ingens*.

coastal areas (Whitaker, 1981), and then post-larvae begin to move from coastal areas into estuaries (Williams, 1964). After spending about 3 months on the nursery grounds, the sub-adult shrimp move back to offshore waters at a total length of about 80–90 mm TL (Larson et al., 1989). Also in Antalya, young shrimps of *P. aztecus* may use

estuaries formed by the Aksu and Köprüçay streams as feeding grounds.

In the Gulf of Carpentaria (Australia), over 97% of adult bopyrid isopods found on commercial penaeid prawns were *E. ingens ingens* (Owens and Glazebrook, 1985), and fourteen different copepod intermediate hosts

were identified by attached cryptonisci or micronisci of these bopyrid isopod species (Owens and Rothlisberg, 1995). The number of larvae was less than 10/m<sup>-2</sup> at shallow depth (8 m), increased to 35 /m<sup>-2</sup> at 25 m, and up to 80 /m<sup>-2</sup> at 55 m with the increase in depth. Greater numbers of cryptonisci were found in deeper water, especially > 40 m (Owens and Rothlisberg, 1995). According to authors with differing views (Owens and Glazebrook, 1985; Jay, 1989), the epicaridium (hatching) larvae of bopyrid isopod parasites must find and infect brackish-water calanoid copepods, their intermediate hosts. Metamorphosed cryptoniscus larvae then quickly detach from the copepod and searches for a definitive host, and then shrimps evidently become infected when they are juveniles in the estuaries or shallower water.

In our study; i) the lower infection rate among juvenile shrimps (CL < 28 mm) than larger and sexually mature shrimps, ii) absence of infected shrimp smaller than 20 mm CL, iii) the low r<sup>2</sup> (0.365) value of the relationship between host size versus the size of the female parasite, iv) the presence of bopyridium or small female parasites in all length classes of host shrimps and v) the presence of a female parasite with 5.8 mm TL in the largest infected shrimp specimen (54.5 mm CL) in March 2020, may indicate that juvenile *P. aztecus* are not infected as late-stage zoeal larvae or post-larvae in the estuarine nursery area or shallower water, rather, initial infection could occur throughout their lives in the offshore. Some studies (Beck, 1980; Owens and Glazebrook, 1985; Owens and Rothlisberg, 1995) support our hypothesis.

Although *P. semisulcatus* has been found as the most commonly parasitized host by *E. ingens ingens* (Rajkumar et al., 2011), in this study, a large number of *P. semisulcatus* and other five penaeid shrimps (*P. japonicus*, *M. monoceros*, *P. kerathurus*, *P. hothor* and *M. aegyptia*) were collected from the Antalya Bay, and, except for only one individual of *P. semisulcatus*, none were infected. A similar phenomenon has been observed in other bopyrids (Warren, 1974; Owens and Glazebrook, 1985; Jay, 1989; Pazir et al., 2016). Owens and Glazebrook (1985) suggested that cryptonisci larvae of the *E. ingens ingens* selectively search for the host prawn *P. semisulcatus* and only infest other shrimps if *P. semisulcatus* is not found within a reasonable period of time. In the coastal waters of Queensland (Australia), two shrimp species (*Penaeus esculentus* Haswell, 1879 and *P. semisulcatus*) by *E. ingens ingens* and other two shrimps (*Penaeus longistylus* Kubo, 1943 and *Penaeus latisulcatus* Kishinouye, 1896) were infected with *Epipenaeon japonica* (Thielemann, 1910), while the other two species (*Penaeopsis endeavouri* Smith, 1962 and *Penaeus ensis* De Haani 1844) were not infected at all (Courtney, 1991). Courtney (1991) explains the result that “although speculative, it is assumable that host-

parasite associations are determined by a combination of host-species selectivity and habitat preference displayed by the infecting cryptoniscan larvae”. These suggestions can explain why phenomenally only *P. aztecus* was infected in our study area. However, this proposition fails to provide an answer about the cause of host-species selectivity. Gopalakrishnan et al. (2017) say that the higher infection of another bopyrid isopod *Palaegyge buitendijki* (Horst, 1910) in female rather than male prawns (*Palaemon malcolmsonii* H.Milne Edwards, 1844) might be due to female having more fatty acids reserves for reproductive purposes. As Gopalakrishnan et al (2017) said, it might be that the chemical composition of the *P. aztecus* in host-species affects selectivity.

Current knowledge related to biological parameters for *P. aztecus* and other seven infected shrimp species by *E. ingens ingens* obtained by various authors in the world is given in Table 3.

In several other studies presenting the monthly prevalence of *E. ingens ingens*, it varied between 0 and 2.9% for *Penaeus monodon* Fabricius, 1798 (Ravichadran et al., 2000) and for *Penaeus styliferus* H. Milne Edwards, 1837 (Gopalakrishnan et al., 2009) and between 1.0 and 3.8% for *Metapenaeopsis stridulans* Alcock, 1905 (Sivasubramanian et al., 2016).

In our study, the infestation in *P. aztecus* varied from 0.86 to 84.8% over the months, and according to our results, the most heavily parasitized host species was *P. aztecus* (Table 3).

No significant difference ( $p > 0.05$ ) in the prevalence of infection between the sexes of *P. aztecus* was found. While similar results were found in *P. semisulcatus* in previous studies (Palisoc, 1987; Somers and Kirkwood, 1991). *Epipenaeon ingens ingens* was found to be more prevalent in *M. stridulans* females than males (Rajkumar et al., 2011; Sivasubramanian et al., 2016).

In the present study, no marked change was observed in the weight of infected female shrimps. Some other studies have found a marked change in host weight following infestation with bopyrid parasites (Courtney, 1991; Chu and Leong, 1996), while other studies reported that the parasite species had no significant effect on host weight (Ayub and Ahmed, 2004; Sivasubramanian et al., 2016). Our results, based on measured changes to ovary weight and GSI value in infected females, support the other nine observations (in Table 3) suggesting that *E. ingens ingens* sterilise their shrimp hosts, whereas uninfected females were berried. Because the female parasite feeds on haemolymph and ovarian fluids after piercing the inner cuticle of the host (Bursey, 1978), the ovary of female host *P. aztecus* cannot develop. The petasma developed normal length and shape according to the size of the infested male shrimp.

**Table 3.** Infection localities and effect of bopyrid parasite *E. ingens ingens* on eight penaeid shrimp species in the world.

Area	Host species	No of examined host shrimp			% prevalence by host shrimp			% attached side of host shrimp			TL (mm) of <i>E.ingens</i>		Effect on host shrimp's				Referans
		F	M	F + M	F	M	F + M	R	L	D	F	M	TW	TL	G	b	
E-India	<i>Penaeus indicus</i>	5*	3*								11-20	3-7					Devi, 1982
S-Turkey	<i>Penaeus semisulcatus</i>	2*									27						Bourdon, 1968
S-India	<i>Penaeus semisulcatus</i>	7250	7227	14527	4.7	0.22	3.4				12-24	4-8			yes		Thomas, 1977
E-Australia	<i>Penaeus semisulcatus</i>					more	2.9										Owens and Glazebrook, 1985
Philippine	<i>Penaeus semisulcatus</i>						0.5									+	Palisoc, 1987
E-Australia	<i>Penaeus semisulcatus</i>			418			1.43						no		yes		Courtner, 1991
Persian Gulf	<i>Penaeus semisulcatus</i>			735**			26								yes		Pazir et al, 2016
N-India	<i>Metapenaeopsis stridulans</i>	215	150		19.3	27.4	24.1								yes		Thirumilu, 2011
SE-India	<i>Metapenaeopsis stridulans</i>			3596	more		1.7				13-22	4-7	no		yes		Rajkumar et al., 2011
SE-India	<i>Metapenaeopsis stridulans</i>			4354	more		1.0-3.8	66.1	38.9		13-22	4-7	no		yes		Sivasubramanian et al., 2016
S-India	<i>Penaeus styliferus</i>			3717	more		1.8	55.9	44.1		13-22	4-7			yes		Gopalakrishnan et al., 2009
N-India	<i>Penaeus styliferus</i>	156	116		30.2	16.7	19.8						no	no	yes		Thirumilu, 2011
N-Australia	<i>Penaeus merguensis</i>			3018			0.89										Owens, 1983
E-Australia	<i>Penaeus esculentus</i>			296			1.35						yes		yes		Courtner, 1991
SE-India	<i>Penaeus monodon</i>			-			0-2.9						yes	yes	yes		Ravichandran et al., 2000
S-Turkey	<i>Penaeus aztecus</i>	40*	30*								18-26	8-10			yes		Korun et al., 2013
S-Turkey	<i>Penaeus aztecus</i>	383	381	784	7.3	7.6	7.5										Teker, 2016
S-Turkey	<i>Penaeus aztecus</i>			515			59.02								yes		Gökoğlu et al., 2020
S-Turkey	<i>Penaeus semisulcatus</i>			124	0	0.01					17	5					This study
S-Turkey	<i>Penaeus aztecus</i>	1421	1582	3043	18.6	16.4	17.5	38.2	43.8	18.0	2-27	2-9	no	yes	yes	+	

(F: female; M: male; R: right; L: left; D: double; G: gonad; no: no effect; yes: effected; +: increased effect; \*: only infested specimen sampled; \*\*: kg).

Interestingly, the maximum size of female *E. ingens ingens* was reported to be 26–27 mm in two other studies in the Mediterranean (Bourdon, 1968; Korun et al., 2013), while the TL of the parasite were much lower (20–22 mm) from India (Devi, 1982; Gopalakrishnan et al., 2009; Rajkumar et al., 2011; Sivasubramanian et al., 2016) (Table 3). To the best of our knowledge, the parasitic isopod *E. ingens ingens* had no previous sizes at first accompaniment and at maturity estimates and no information on developing embryos and newly hatched epicaridum larvae. In addition, a double infection on the host shrimps was not reported in any of the other studies.

In all previous studies (except Owens, 1983) in Table 3, no information on the reproductive activity and fecundity of the female parasite *E. ingens ingens*. Similar to our results, female *E. ingens ingens* spawns for the first time at approximately 10 mm TL, and 10 000–110 000 epicaridum larvae were produced by one adult female *E. ingens ingens* in one brood in the Gulf of Carpentaria (Owens, 1983).

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In conclusion, the parasitic guest *E. ingens ingens* is one of the prime threats to the commercial fisheries of host shrimp *P. aztecus* in the Antalya Bay, and it also affects the commercial value of the host, affecting its reproductive potential in the wild. In addition, the distribution of the parasitic guest *E. ingens ingens* in the Mediterranean may expand like the spread of its host shrimp *P. aztecus* in the future.

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## Conflict of interest

The authors declare that they have no conflicts of interest.

## Ethics approval

No ethical approvals are required.

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