

## Research Article

## Temperature and salinity tolerance of the larval stages of the African pea crab *Afropinnotheres monodi* Manning, 1993: implications for its dispersal along European waters

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### Abstract

The marine parasitic African pea crab *Afropinnotheres monodi* Manning, 1993 has well-established populations in the gulf of Cadiz, where it is invasive. In this study, its tolerance to temperature and salinity variation during larval development were studied in the laboratory. Larvae were reared in seawater (salinity 36) at different temperatures (5, 10, 15, 20, 25 and 30 °C). At 20 and 25 °C larval development was successfully completed, with the highest rate of survival to the megalopa stage occurring at 25 °C. At this optimal temperature larvae were reared at different salinities (15, 25, 36 and 45), and maximum survival to megalopa occurred at salinity 25. Additionally, the time required for larval developmental increased with salinity: the shortest (12 days) and the longest (25 days) development time occurred at salinities 25 and 45, respectively. In some of the treatments an extra stage (zoea V) was observed, but the only zoea V specimens that successfully moulted to megalopa were those reared at 25 °C and salinity 25. Temperature seems to be the main factor determining whether larval development is successfully completed, whereas salinity has an additional effect on duration of larval development under optimal temperature conditions. Our results suggest that *A. monodi* is well-adapted to withstand higher temperatures than currently present in Europe's marine waters and, if temperatures keep increasing, this species may be able to expand successfully northward along the European Atlantic and Mediterranean coasts.

**Key words:** Pinnotheridae, zoea, megalopa, invasions, tropicalization process

### Introduction

Numerous marine species have shifted their ranges poleward in response to warming from global climate change (Hale et al. 2017). Several African species, including *Afropinnotheres monodi* Manning, 1993, are moving northward and invading the South European coasts, probably due to global warming. Recently, Perez-Miguel et al. (2019) reported the expansion of this species' range into the Northern Atlantic Ocean in Portugal (Setubal) and into the eastern part of the Mediterranean Sea (Malaga, Spain). The

first record of this species in the southwest of Europe was in 1995 in the Bay of Cadiz (SW Spain), as *Afropinnotheres* sp. (López de la Rosa et al. 2002); and later it was identified as *A. monodi* and recorded in several different localities of the Gulf of Cadiz (Subida et al. 2011).

Not much information exists about survival and duration of larval development of any species in this family under different combinations of temperature and salinity. This lack of knowledge is probably due to their small size and symbiotic lifestyle (Palacios-Theil et al. 2009; Becker and Türkay 2010). Pinnotheridae De Haan, 1833 is a brachyuran crab family characterized by having symbiotic relations with invertebrates, mainly bivalves (Schmitt et al. 1973). They are heavily dependent on the host species and spend most of their life inside bivalves. In the case of *A. monodi*, juveniles and adults have been found inside specimens of 10 different bivalve species in intertidal habitats (Perez-Miguel et al. 2019). This African pea crab may have a negative effect on the condition status of their bivalve hosts. In the case of *Mytilus galloprovincialis* Lamarck, 1819, when the parasite crab was an ovigerous female of *A. monodi*, the condition index of mussels decreased 14.7% on average (Perez-Miguel et al. 2018). Besides, in some areas of the Algarve (South of Portugal) cockles (*Cerastoderma* spp.) were sometimes hosting *A. monodi* at 100% of prevalence; this high infestation made that a canning company has closed the canning line of cockles due to economic losses (Cuesta, *pers. comm.*).

Like other marine brachyuran crabs, the larval phase of the pinnotherids consists of a planktonic period with 2–5 zoeae and a megalopa stage (Pohle and Marques 1998; Bolaños et al. 2005). Specifically, the larval development of *A. monodi* consists of four zoeal stages and a megalopa stage (Marco-Herrero et al. 2016). These planktonic stages are considered to be the main dispersive phase of the African pea crab. Currently, the invasive stage at which *A. monodi* enter their bivalve hosts is unknown. The first crab instar could be the invasive stage of this species (Perez-Miguel 2018). The juvenile crab mainly infests small-size bivalves such as *Cerastoderma edule* (Linnaeus, 1758). Males and hard females can change hosts, but they must spend most of the time inside the host since they were not habitually collected in the water column (Drake et al. 1998). Reproductive females (soft females) reach larger size than males and are normally found within larger bivalves such as mussels. These soft females cannot leave the host as they are obligate parasites.

The spatial distribution of marine organisms is determined mainly by their physiological characteristics, environmental factors (e.g. temperature and salinity), their dispersal potential and ecological interactions (Giménez and Anger 2001; Giménez 2010; Podbielski et al. 2016; De Grande et al. 2018). Among environmental factors, the influence of temperature and salinity on decapod crustacean larvae has been extensively documented (Anger et al. 1989; Mohamedeen and Hartonoll 1989; Sulkin and McKeen

1989; Werding and Müller 1990; Harms 1991; Gonçalves et al. 1995). Temperature and salinity are considered the key environmental stressors affecting recently-arriving species (Lee et al. 2003). Salinity affects larval biology, including survival, morphology, moult cycle, feeding, metabolism, behaviour, and development (Anger 2003). Water temperature also has considerable influence on survival, growth and development rates, as well as on population dynamics (Anger 2001, 2016). Thus, in non-native species, a high tolerance to variation in these environmental factors can be important in their successful establishment (Podbielski et al. 2016). Understanding the tolerance of non-native species to environmental conditions is crucial to understanding their potential spread (Forsström et al. 2018). However, no published studies have examined how climate-driven changes of temperature and salinity are affecting the larval ecology of this species and influencing its potential range shifts in nearshore waters. A preliminary experimental study by Marco-Herrero et al. (2016) described the effect of temperate temperatures (19 and 25 °C) on survival of *A. monodi*, and the duration of its larval development was related to the dispersal capability of this species.

The aim of this study is to assess the environmental range in which the larval development of *A. monodi* is successful; and from this, to evaluate the possibilities of a potential infestation of North European populations of bivalves of important commercial interest that act as hosts of this African pea crab. We conducted a series of experiments to evaluate effects of temperature and salinity on larval survival and duration of development of this African species. Our hypothesis was that the non-native species *A. monodi* displays an optimum at higher temperature than those found in the northern temperate zone, and global warming may facilitate its expansion northward along the European Atlantic and Mediterranean coasts.

## Materials and methods

Ovigerous females of *A. monodi*, hosted by intertidal specimens of the mussel *M. galloprovincialis*, were collected in the Gulf of Cadiz (Southwestern Spain) in February, May, and September 2016 and maintained in the laboratory until hatching. Hatched larvae were reared at different temperature and salinity conditions to assess the more suitable combination of these environmental factors on larval survival and development duration. The response variables were percentage survival and mean duration of larval development and each zoeal stage. To simulate natural conditions inside hosts, ovigerous females were maintained individually in 1 L plastic vessels with aerated water and a piece of plastic tube as a shelter at salinity 36. Females were fed *ad libitum* with fish food flakes until the larvae hatched. Twice a day, aquaria water was changed (8 am and 6 pm) to evaluate the embryonic stage.

Freshly-hatched larvae were concentrated using a point-light source and transferred to individual plastic vessels (10 mL) using a sterile pipette at each of the temperature and salinity combinations. Only zoeae of the same hatch within a maximum ~ 10 h after hatching were selected for experiments. Water in individual vessels was changed daily. Larvae were fed *ad libitum* with the rotifer *Brachionus plicatilis* Müller, 1787 that had been fed with *Nannochloropsis gaditana* L.M. Lubián, 1982 and *Isochrysis galbana* Parke, 1949. Larvae were checked daily for moulting or mortality under a stereo microscope.

The experiments continued until all the larvae died or moulted to megalopa. The criteria for the death confirmation were the total lack of movement of appendages, the immobility of the hearth and the opaque white body color. When necessary, death of larvae was confirmed using a microscope (Axioskop 50, Carl Zeiss).

Firstly, in order to know the optimal range of temperature for development, experiments used at a constant salinity (36) and a wide range of temperatures (5, 10, 15, 20, 25 and 30 °C [ $\pm 0.1$  °C]). Prior to experiments, larvae were reared in individual containers of 10 mL with sea water at the temperature they were collected in the field and were distributed in the incubators according to the experimental temperatures. Freshly-hatched larvae from three females collected in February 2016 (field temperature  $\approx 15$  °C) were used to test a range of lower temperatures (5, 10 and 15 °C), whereas hatched larvae from three females collected in May 2016 (field temperature  $\approx 22$  °C) were used to test a range of higher temperatures (20, 25 and 30 °C).

Once the optimal temperature for the larval development of this species was known (25 °C), the experiments to study the effects of salinity on larval development were carried out at this optimal temperature and salinities of 15, 25, 36 and 45. These four different salinities were chosen because they are within the range of salinities that can occasionally occur in the habitats inhabited by the hosts of the target species. The salinity experiments were carried out simultaneously in time and using larvae from females collected in September 2016 (field temperature  $\approx 25$  °C).

For each experimental temperature and salinity tested, 90 larvae from three different females (30 larvae per females) were used. To prevent excessive stress, larvae were acclimated to the final temperature or salinity conditions of each treatment by increasing or decreasing temperature and salinity gradually (2.5 units every 12 hours) until reaching experimental conditions.

Furthermore, to test the healthy condition of newly hatched zoeae from different females and to determine whether females showed similar condition, 12 larvae per female were cultivated, under starvation, at 20 °C and salinity 36. All experiments were conducted inside culture chambers (Ibercex-F1) with constant temperature and photoperiod (12hL/12hD).

The seawater used in experiments was from Bay of Cadiz (36°29'39"N; 6°13'01"W) which was filtered with a Vacuum Filtration System and glass microfiber filters. Experimental water with higher salinity (salinity 45) was obtained by dissolving artificial sea salts (Instant Ocean®) and that with lower salinities (15 and 25) by diluting seawater with distilled water. Experimental salinities were confirmed using a refractometer (V<sup>2</sup>Refractometer®).

### *Statistical analysis*

Responses of survival over time were analysed with the Kaplan-Meier log-rank test within the R survival and survminer packages considering the censored observations along the larval time (Kassambara and Kosinski 2017). The median Lethal Time (LT<sub>50</sub>), the statistical average time during which 50% of a given population dies, was estimated with the function “surv\_median” from the package survminer. Posthoc survival analysis was evaluated with the function “pairwise\_survdiff” from the package survminer. *P*-value stands for the log-rank test that compared whether survival curves were different from the null hypothesis.

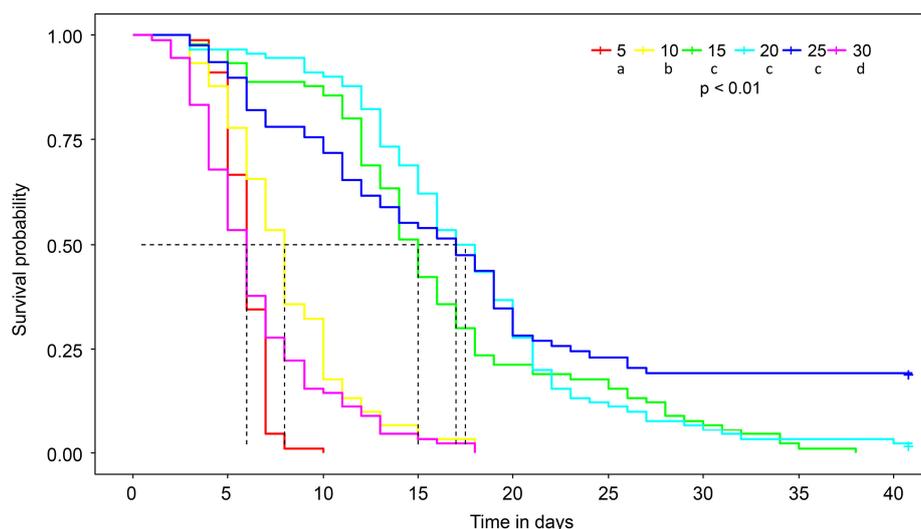
The total duration of larval development and each larval stage were tested through generalized linear mixed model GLMM. In both experiments, the designs were factorial; in the temperature experiment, there were two factors “temperature” (fixed factor) and “female” (random factor). In the salinity experiment, “salinity” was one fixed factor and “female” one random factor. The replicate unit for the GLMM was the individual larvae. All statistical analyses were performed using R software version 3.4.0 (R Core Team 2017). A significance level of  $P \leq 0.01$  was considered for all statistical tests carried out in this study.

## **Results**

### *Temperature treatment*

Larvae successfully developed to megalopa in only two out of the six temperature treatments to which they were exposed, 20 and 25 °C. At 25 °C the average survival rate of the overall larval cycle was 19.2%, while in 20 °C was 2.2%. Considering the survival values *versus* time, results fall into two clear groups (Figure 1): a first group, comprising larvae reared at 15, 20 and 25 °C, showed significantly higher survival values ( $P \leq 0.01$ ). A second group, comprising larvae reared at 5, 10, and 30 °C, showed significantly lower survival values ( $P \leq 0.01$ ). In the first group, the highest LT<sub>50</sub> value was observed at 20 °C, followed by 25 °C and 15 °C (Table 1). In the second group, the lowest LT<sub>50</sub> and average survival values were recorded for larvae reared at 5 and 30 °C, followed by 10 °C (Figure 1).

When survival was analysed at each zoeal stage, low temperatures produced a significant reduction in survival. At temperatures of 5 and 10 °C none of reared larvae moulted to zoea II, while for larvae reared at 15 and



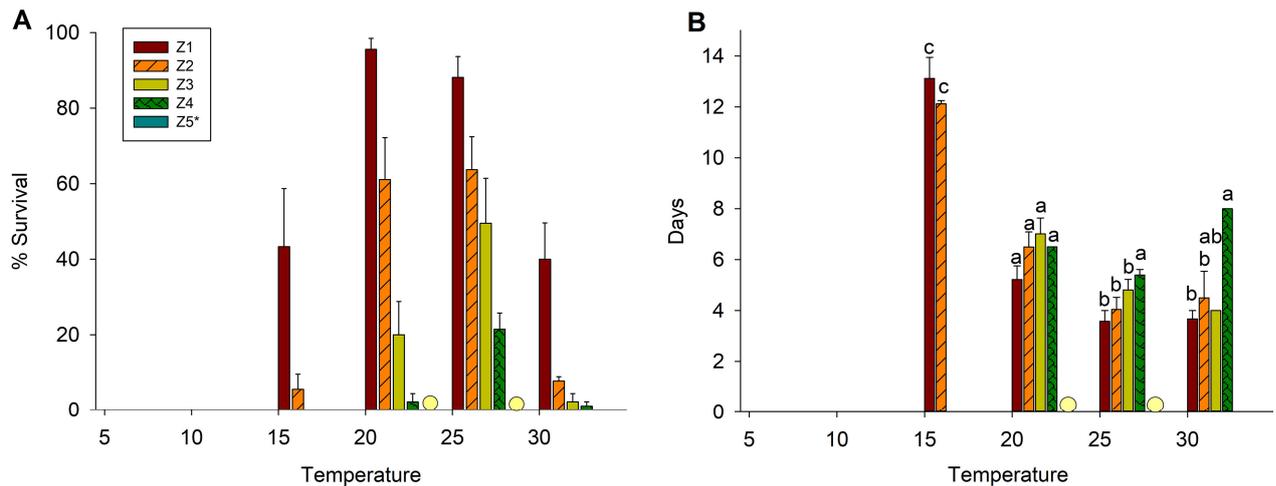
**Figure 1.** Survival probability at different temperatures during the larval development of *Afropinnotheres monodi* under culture conditions. The larvae that molt to megalopa stage were considered as not dead until the end of the experiment. The  $LT_{50}$  is depicted as a dashed black line. The letters correspond to treatments with significant difference ( $P \leq 0.01$ ). The statistical method was the Kaplan–Meier log-rank test within the R survival and survminer packages considering the censored observations along the larval time.

**Table 1.** Number of zoeae and megalopae and the median Lethal time ( $LT_{50}$ ) of *Afropinnotheres monodi* in the different treatments of temperature and salinity. (\*) Zoea V stage number represents the total number of extra zoea V that was obtained in the different experiments; (\*\*) the only case in which one out of the two zoea V molted to megalopa.

Treatment	$LT_{50}$ (days)	Zoea I Started	Zoea II	Zoea III	Zoea IV	Zoea V*	Megalopa
Temperature (°C)							
Starvation	6.25	70	0	0	0	0	0
5	6	90	0	0	0	0	0
10	8	90	0	0	0	0	0
15	15	90	39	5	0	0	0
20	17.5	90	86	55	18	0	2
25	17	78	70	50	40	1	15
30	6	90	36	7	2	1	0
Salinity							
Starvation	5.5	36	0	0	0	0	0
15	6	90	9	3	0	0	0
25	19	90	80	69	63	2**	42
36	26.5	90	76	61	56	2	43
45	12.5	89	54	27	20	1	16

30 °C, less than 45.0% moulted to zoea II (Figure 2 and Table 1). However, for larvae reared at intermediate temperatures (20 and 25 °C) more than 89.0% moulted to zoea II (Figure 2A). From the zoea II to megalopa, the highest survival rate (21.4%) was at 25 °C. At this temperature, 37.5% moulted from zoea IV to megalopa. Larval exposure to 20 °C resulted in a significant reduction of survival, with only 2.3% of larvae moulting from zoea II to megalopa, and 11.1% moulting from zoea IV to megalopa. At 30 °C, 40.0% moulted to zoea II, and only 5.5% moulted from zoea II to zoea IV.

Duration of total larval development at the two temperatures at which larvae reached megalopa stage was markedly shorter at the highest temperature: 17 and 24 days, at 25 and 20 °C, respectively (Figure 2B and Supplementary material Table S1: Model 1). The development time required



**Figure 2.** (A) Temperature effects on zoeal stages survival, mean  $\pm$  standard error (%), and (B) mean development duration  $\pm$  standard error of zoeal stages in the different temperature treatment of *Afropinnotheres monodi* cultured larvae. Yellow dots mean the treatment finished when reaching megalopa stage. Letters indicate significance differences among experimental temperature levels at each zoeal stage (GLMM:  $P \leq 0.01$ ). Similar letter indicates non-significant differences among treatments at each zoeal stage.

for each larval stage was also shorter at these two temperatures, and there was a tendency to spend more days in the late zoeal stages than in the early ones.

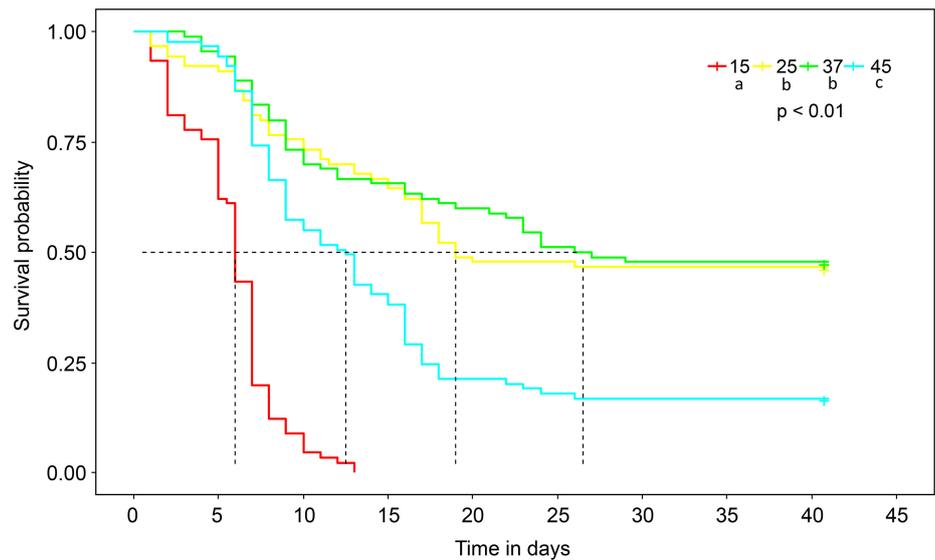
The effects of temperature on the total duration of development of *A. monodi* were observed mainly on the intermoult duration; this was longer in the zoea I and II stages of larvae reared at temperatures of 15 and 20 °C, compared to those reared at 25 and 30 °C (Figure 2B). Interestingly, some larvae reared at temperatures of 25 and 30 °C developed through an extra stage (zoea V) although they did not moult to megalopa (Table 1). When larval development time under starvation was assessed as a proxy of the maternal fitness,  $LT_{50}$  values of zoeae from different females did not show any significant differences (Table S2: Model 3). Mean  $LT_{50}$  values of newly-hatched zoeae in starvation condition (at 20 °C and salinity 36) was 6.35 days, ranging between 5.5 and 7.5 days.

### Salinity treatment

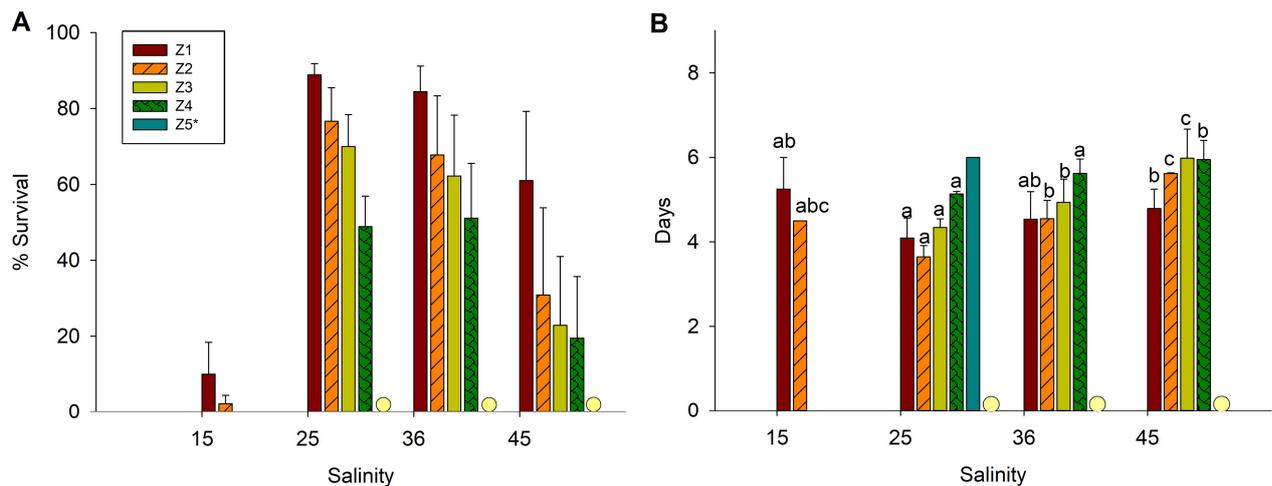
At 25 °C, the experimental salinities of 25, 36, and 45 resulted in maximum survival rates of larvae reaching the megalopa stage, especially at salinity 25 and 36 ( $P \leq 0.01$ : 46.6% and 47.7%, respectively; Figure 3). However, at salinity 15, larvae did not complete their development successfully (they only reached the zoea II stage) and showed the lowest  $LT_{50}$  values (Table 1).

When survival at each zoeal stage was analysed, the lowest and the highest experimental salinity levels tested (15 and 45) produced a significant reduction in survival: only 3.3% of larvae reached zoea III stage at the lowest salinity, and only 18.0% moulted to megalopa, at the highest salinity. However, more than 45.0% of larvae reared at salinity 25 and 36 moulted to the megalopa stage (Figure 4A).

Although the effect of salinity on the duration of development was weaker than that of temperature, a significantly longer duration was observed



**Figure 3.** Survival probability at different salinities during the larval development of *Afropinnotheres monodi* under culture conditions. The megalopa stage was considered as alive until the end of the experiment. The  $LT_{50}$  is depicted as a dashed black line. The letters correspond to treatments with significant difference ( $P \leq 0.01$ ). The statistical method was the Kaplan–Meier log-rank test within the R survival and survminer packages considering the censored observations along the larval time.



**Figure 4.** (A) Salinity effects on zoeal stages survival mean  $\pm$  standard error (%), and (B) mean development duration  $\pm$  standard error of zoeal stages in the different salinity treatments, of *Afropinnotheres monodi* cultured larvae. Yellow dots means the treatment finished when reaching megalopa stage. Letters indicate significance differences among experimental salinity levels at each zoeal stage (GLMM:  $P \leq 0.01$ ). Similar letter indicates non-significant differences among treatments at each zoeal stage.

in larvae reared at higher salinities (Table S1: Model 2). The average duration of larval development was 18.3, 18.9 and 20.4 days for salinity 25, 36 and 45, respectively; whereas at salinity 15 all larvae died at zoea III stage (Figure 4B).

At each successive stage, the intermoult duration tended to be longer from lower to higher salinity. At salinity 45, intermoult duration was significantly longer compared to the duration observed at salinity 25 and 36, in zoea III and zoea IV stages. In addition, in larvae reared at salinity 25, 36 and 45 an extra zoea V stage was observed, although moulting from that stage to megalopa only occurred successfully at salinity 25 (Table 1).

The starvation experiment to assess fitness of the three ovigerous females showed a mean  $LT_{50}$  value of 5.5 days, ranging between 4 and 7 days. In this case, the  $LT_{50}$  value of zoeae from one of the three females was significantly lower (Table S2: Model 4,  $P \leq 0.01$ ) than the values of zoeae from the other two females.

## Discussion

The pea crab *A. monodi* is an African species that reached European coasts about 20 years ago. It has demonstrated an invasive behaviour by expanding its range in the Northern Atlantic Ocean, which was probably facilitated by global warming (Subida et al. 2011; Perez-Miguel et al. 2019). African species are reaching European coasts by natural northward expansion (Vermeij 2012; García Raso et al. 2014), a process known as tropicalization, as well as by human-mediated means (Cuesta et al. 2016). Dispersal and establishment of new populations of *A. monodi* depend on the influence of several abiotic and biotic factors on the different stages of its complex life cycle. Among environmental factors, temperature and salinity are considered two key stressors affecting the spread of species to new localities (Lee et al. 2003). In crab species, the planktonic larval phase is considered to be the main dispersive phase (Cowen and Sponaugle 2009) but for some pea crab species planktonic larvae as well as juveniles are dispersive stages (Møller and McDermott 1958). In the Bay of Cadiz, zoeal and megalopal stages of *A. monodi* are caught in the water column but not juveniles (Drake et al. 1998). In fact, the juvenile hard stage was collected inside *A. monodi* hosts, mainly intertidal benthic bivalve species as *Cerastoderma* spp. and *Scrobicularia plana* (Drake et al. 2014). Thus, dispersal success of the species to new localities depends on their five larval stages (four zoeae and one megalopa).

As previously found in other marine invertebrates (e.g. Marco-Herrero et al. 2016; Mak and Chan 2018), experimental results of the present study indicate that temperature has a significant effect on the survival and duration of the planktonic larval phase of *A. monodi*. When larvae were reared in seawater at the two coldest temperatures tested in our study (5 and 10 °C) they presented  $LT_{50}$  values similar to those of larvae in starvation treatment (20 °C), suggesting that the larval activity was reduced and that they were not able to feed correctly. Thus, larval survival decreased. Indeed, larvae reared at 5 °C remained alive for several days but they did not show any movements, whereas those reared at 10 °C displayed some movements but these were fewer and slower than those of larvae reared at warmer temperatures (above 15 °C). Thus, the observed limited mobility at low temperatures may prevent larvae from capturing prey and causing them to die by starvation.

When larvae were reared at temperatures between 15 and 30 °C, although the development time was shorter at higher temperatures, the survival rate

observed had an inverted u-shaped pattern. This suggests the optimal temperature range for larval development is between 20 and 25 °C. At both of these temperatures, some larvae completed their development to the megalopa stage, although the proportion of larvae reaching this stage was considerably higher at 25 °C (19.2%). A reduced survival rate was observed when larvae were exposed to suboptimal temperatures, both high and low.

The effects of temperature on moulting led to a more rapid development when water temperatures were higher. The difference in development time required for each zoeal stage at 15 and 30 °C indicated effects on the intermoult duration, suggesting an increased metabolism during larval moulting at higher temperatures (Wenner 1985; Brown et al. 1992). Therefore, the low survival rate and short development time of larvae reared at 30 °C suggests an excessive acceleration of the metabolic rate with the consequent thermal stress (e.g. González-Ortegón and Giménez 2014). In contrast, the low survival and long development time observed for larvae reared at 15 °C suggest a delay in the development stage, as if they need warmer temperatures, triggering an increase of larval dispersion. In other words, even if the larval development of *A. monodi* can take place within a relatively wide range of temperatures, exposure of larvae to suboptimal environmental conditions of temperature may cause different kinds of deleterious metabolic alterations (Harms 1991) and a collapse of higher physiological functions (Pörtner 2001).

When salinity tolerance of *A. monodi* was tested at an optimal temperature (25 °C), the pea crab was able to complete larval development at salinities between 25 and 45. However, the higher percentage of larvae reaching the megalopa stage at salinities of 25 (46.7%) and 36 (47.8%) suggests that *A. monodi* must be included in the group of stenohaline marine crabs (Castejón et al. 2015). There is controversy about which is the predominant factor that determines the development time and survival in crustacean larvae. For some authors, salinity is the most significant factor (Anger 2003; Castejón et al. 2015), whereas for others it is temperature (Harms 1991). In our study, temperature was the factor with major influence on development duration and survival of *A. monodi* larvae. However, the fact that in brackish water (salinity 15) larvae of this species did not reach the zoea III stage, even at the optimal temperature of 25 °C, puts in question its likelihood of extending its presence into some estuarine habitats inhabited by some of its hosts (Perez-Miguel et al. 2019). In any case, the survival of the first two zoeal stages at this salinity indicates that there is enough time for larvae hatching in estuaries to be exported to the sea and complete their development there.

Both survival and duration of larval development under different environmental conditions may be affected by the fitness and phenotypic characteristics of parental females (Plaistow and Benton 2009; González-Ortegón and Giménez 2014). Values of both response variables for larvae

under the starvation treatment indicated that, for temperature treatments, in the case of the two groups of parental females there were no significant differences between them in the fitness of newly hatched larvae. Therefore, the maternal effect can be eliminated from factors causing the observed differences between different temperature treatments. However, the variability of response observed among parental females of each group at each temperature could be due to differences in genotypic ability to tolerate stressing temperatures (Faria et al. 2017). Conversely, there was a significant difference in larval survival among parental females used for the salinity experiment. Differences in variability between females can have different causes such as differences in feeding that leads to a higher quality in the biochemical composition of larvae (Giménez et al. 2004). Nevertheless, since we used larvae from the three females at the four salinities tested, the effect of the female's characteristics could not be causing the observed salinity-related differences.

The results of this study suggest that the larval tolerance of *A. monodi* to different temperatures are in agreement: a) with the temperature-dependent seasonal pattern that has been observed in the abundance of ovigerous females in field; b) with the sea water temperature and salinity all throughout the year in the current geographical distribution of the species (Perez-Miguel et al. 2019). Coastal seawater temperatures in the continental shelf of the south-west of Europe display a clear seasonal pattern, with monthly mean temperatures oscillating from  $\approx 15 \pm 1$  °C in January-February to  $\approx 25 \pm 1$  °C in July-August (Navarro and Ruiz 2003; García-Lafuente et al. 2012). Accordingly, in the Bay of Cadiz ovigerous females of *A. monodi* were found all year round but with an autumnal decrease of the reproductive activity and a subsequent decrease of larval abundance in winter (Drake et al. 1998, 2014). In contrast, the three native European pinnotherid species inhabit colder waters (Becker and Türkay 2010; Perez-Miguel et al. 2019). That is, *Nepinnotheres pinnotheres* with the northern limit of their distribution in Ireland, *Pinnotheres pectunculi* on the Brittany coast and *P. pisum* on the Norwegian coasts. However, although the southern limits of distribution recorded for these European species overlap with the current northern limit of distribution of *A. monodi*, native European species have only been found with very low prevalence in intertidal populations of bivalve species used as hosts by *A. monodi* (Lynch et al. 2014; Becker and Türkay 2017; Perez-Miguel et al. 2019). Thus, differences in the optimal temperatures for larval development of the European pinnotherids and of the African species studied, together with different habitat preferences, may have given rise to a segregated use of some common hosts, which has facilitated the successful establishment of *A. monodi* in the south of the Iberian Peninsula. Furthermore, experimental assays of *A. monodi* adult survival at different temperatures indicated they have a wider range of temperature tolerance (10–30 °C) than larvae (*unpublished data*), which permit them to live in the more stressful intertidal habitat.

As *A. monodi* is obligate symbionts of bivalves, its expansion further north requires the presence of its hosts at higher latitudes. Since there are mussel and cockle populations all along the Atlantic European coasts, the host availability would be not a limiting factor of the expansion of this species northwards. Considering the data obtained in the present study, the capability of *A. monodi* to invade northern Atlantic populations of its bivalve hosts by natural larval dispersal is currently very limited by temperature. Even if larvae or adults are introduced by human-mediated means (e.g. in ballast water, fouling or transplantation of infested mussels or dog cockles) in these northern populations beyond Setubal (Portugal; the current northern limit), the possibility of their successful colonization by *A. monodi* would be limited by the colder waters in the northwest of the Iberian Peninsula (12–18 °C) leading to a short reproductive period (Gago et al. 2011).

Nevertheless, future warming of coastal waters is likely to facilitate the future dispersion of this species northwards. Other invasive species, such as the ascidian *Microcosmu exasperatus* Heller, 1878, are expected to spread further into new locations due to the relative temperature and salinity tolerance range together with the anticipated increase in anthropogenic disturbances (Raijman and Shenkar 2016). In addition, a recent thermodynamic model, developed to estimate the optimal temperatures for crustacean larval survival/growth, postulated that, for a given species, the optimal temperature changes in accordance with the geographical distribution of the species (Yamamoto et al. 2017). For example, optimal survival temperature of the first zoea of *Portunus pelagicus* is 20.5 °C for their South Australia populations (Bryars and Havenhand 2004), but between 26 and 28 °C for their populations in the western Peninsula of Malaysia (Efrizal 2016). Thus, it is possible that optimal temperatures for larval survival in the case of the northern Setubal (Portugal) population of *A. monodi* are lower than those determined for larvae from the Gulf of Cadiz population. Synergy between these two aspects mentioned, a scenario of increased temperatures and a latitude-dependent optimal temperature for larval development and acclimation of *A. monodi*, may result in the species soon reaching the mussel aquaculture areas of the NW Iberian Peninsula (Galicia), with the consequent negative impact on mussel cultures due to the loss of condition of the mussels infested with this African parasite (Perez-Miguel et al. 2018).

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### Supplementary material

The following supplementary material is available for this article:

**Table S1.** GLMMs to evaluate duration of total larval development in individuals reared under different temperatures (Model 1) and salinities (Model 2).

**Table S2.** GLMM to evaluate larval development time under starvation as a proxy of the maternal fitness.

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