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Salinity effects on the first larval stage of the invasive crab *Hemigrapsus takanoi*: Survival and swimming patterns

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ABSTRACT

The Asian brush-clawed shore crab *Hemigrapsus takanoi* has high tolerance for environmental changes facilitated the establishment of non-native populations along the Atlantic European coast. The self-maintenance and potential spread of this invasive crab will partially depend on its ability to disperse during the larval period. Larvae are not equipped with efficient osmoregulatory mechanisms to tolerate low salinity conditions; therefore, they evolved specific swimming behavior that facilitates exportation offshore for development in more stable and higher salinity conditions. To study the salinity tolerance, we quantified the survival of newly hatched larvae subjected to salinities ranging from 2 to 35 over a 24 h period. We observed that more than 50% of larvae could survive 24 h only at salinities higher than 20, and that shorter incubation periods of 2–6 h could produce high mortality at salinities lower than 10. We used video-tracking techniques to quantify swimming in newly hatched larvae at different levels of salinity, and under starvation or food availability conditions. The results showed that apparent swimming speed increases as salinity increases, and that upward trajectories are faster than downward ones. When food was available, the larvae reduced the frequency of helical swimming trajectories, turned out to be faster, straighter and more vertical. At salinities lower than 20, the swimming trajectories became more random, and the described patterns tended to disappear. Our results indicate that lower survival and reduced swimming performance may constrain the dispersal capacity of the non-native populations located at low salinity habitats.

1. Introduction

Salinity is a major factor structuring coastal and estuarine ecosystems. Reduced salinities may occur with considerable spatial and temporal fluctuations (Anger, 2003). The species inhabiting such variable ecosystems must have specific adaptations to ensure a stable internal osmotic environment needed to perform all the physiological and metabolic processes needed (Evans, 2008). Thus, euryhaline species are better able to establish non-native populations and become invasive as a result of a broad salinity tolerance.

The Asian brush-clawed shore crab *Hemigrapsus takanoi* is a key benthic species found in low-energy, fine-sediment habitats under intertidal boulders in the inner bays and estuaries of the Northwestern Pacific (Asakura and Watanabe, 2005). Nonindigenous populations have been reported along the Atlantic European coast where *H. takanoi* is now considered an invasive species. It seems that shipping lines may have allowed *H. takanoi* to establish their populations in Europe from multiple introduction events coming from disparate Asian populations using the ballast waters, hulls of ships or sea-chests (Makino et al., 2018). Since 1994, when *H. takanoi* was found for the first time in the Bay of Biscay (Noël et al., 1997), the population has expanded broadly northward until the western Baltic Sea (Ashelby et al., 2017). The ecological impact of this dominant species (together with another invader *Hemigrapsus sanguineus*) is already visible in the local displacement of the native crab *Carcinus maenas* (Landschoff et al., 2013). The successful colonization of diverse habitats may be explained by the

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ability to tolerate a wide range of salinities (Shinji et al., 2009). In fact, we have observed this crab inhabiting river mouths (salinity of 2–5) and open coastal areas (salinity of 34–35) in Tokyo Bay, its type locality, as well as exposed to freshwater events during heavy rain in the intertidal zone during low tide. *Hemigrapsus takanoi*, like the congeners *Hemigrapsus crenulatus* and *H. sanguineus*, are euryhaline species that change their metabolism in response to variations in salinity to regulate their internal osmolality. For instance, these species increase their oxygen consumption, ammonia excretion and regulatory capacity of Na^+ as salinity decrease, as part of their osmoregulatory mechanisms (Shinji et al., 2009; Urzúa and Urbina, 2017; Hudson et al., 2018). The increased energy losses at low salinity due to respiration and excretion are compensated by an increment in the ingestion rate, contributing to their success in estuarine ecosystems (Urbina et al., 2010).

Like most of benthic marine invertebrates, the brachyuran crabs pass through a complex life cycle including a planktonic larval phase and a benthic adult phase. Living freely in the water column exposes the larvae to potential mortality risks such as advection by currents, predation or starvation, and also facilitates the larval dispersal allowing the exchange of individuals among populations (Cowen and Sponaugle, 2009). In this sense, larval dispersal is a major mechanism to expand the distribution range of the invasive species once population is established, as likely has happened with *H. takanoi* throughout the European coast (Makino et al., 2018). In estuarine crabs, larval stages generally do not possess such efficient osmoregulatory mechanisms to tolerate a wide range of salinities as the adult crabs do (Anger, 2003). It has been observed that the increase of energy expenditures for osmoregulation at low salinities affects the larvae in the use of energy reserves, biomass and survival (Giménez and Torres, 2002; Diele and Simith, 2006; Urzúa et al., 2018). In the case of *H. takanoi*, larval development comprises five zoea stages and one megalopa stage and is completed in 25 days (Landeira et al., 2019). The zoea I stage lasts 4 days and the small body size, the absence of lipidic drops, and the fully developed mandibles, maxillule, and maxilla indicate that the larva is obligated planktotrophic. The successful development occurs only in salinities higher than 25 (Mingkid et al., 2006a). Therefore, to allow for significant larval survival and population maintenance, estuarine crabs (like *Hemigrapsus* species) have evolve specific strategies to export larvae offshore for development in more stable and higher salinity conditions; once they reach the final megalopa stage, larvae return to the estuarine habitat for initiating the benthic life stage (Queiroga and Blanton, 2005; Epifanio and Cohen, 2016). The swimming performance and behavioral mechanisms, referred as selective tidal-stream transport, are involved in the larval export. Thus, larvae are able to perform vertical migrations towards the surface in synchrony with ebb tides, resulting in net seaward transport out of the estuary (López-Duarte and Tankersley, 2007). In the case of *H. sanguineus*, experiments in the laboratory have shown that newly hatched larvae are negatively buoyant but the combination of negative geotaxis and positive barokinesis enhance upward swimming keeping them in the surface where the net transport is seaward (Park et al., 2004). However, since salinity has a direct influence on the buoyancy of the larvae and the larvae hatch in diverse conditions of salinity inside the estuary, the *in situ* salinity may constrain the swimming performance, and hence their capacity for being exported and dispersed.

In the present article, we explored in laboratory experiments the effects of salinity on the swimming performance and survival in newly hatched larvae of the Asian brush-clawed shore crab *H. takanoi*. We hypothesize that spawning regions at low salinity conditions may affect the dispersal capacity during the larval stages, and hence the invasiveness of this species.

2. Material and methods

2.1. Collections and maintenance

Ovigerous *Hemigrapsus takanoi* used for this study were collected in Daiba Park, (35°38′04"N, 139°46′26"E), located in the inner side of Tokyo Bay, in the summer of 2017 and 2018 (experiment 1: July 10, 2017; experiment 2: July 17, 2017; experiment 3: August 14, 2018). To reduce the incubation time in the laboratory and minimize the potential maternal effect, only ovigerous crabs with similar size (12 mm carapace length) with embryos in the advanced stage of development (eyes visible) were collected. In the field, the specimens were identified following the key characters of pigmentation pattern on the abdominal somites, and on the ventral surface of the carapace described by Asakura and Watanabe (2005). In the aquarium facilities of the Tokyo University of Marine Science and Technology at Shinagawa Campus, the crabs were placed individually in 1 L plastic buckets containing 0.8 L of 20 °C and 25 salinity seawater (field conditions at the time of collection), with aeration and under natural daylight conditions. Every day, the water was changed, and the crabs were fed pieces of the seaweed wakame *Undaria pinnatifida*. After experiments, the crabs were returned to the collection site.

2.2. Experiment 1. larval survival

On hatching, actively swimming larvae were separated from 3 ovigerous *H. takanoi* using a pipette and transferred to a 5 L bucket. They were gently mixed to ensure that larvae from different females will be evenly distributed across treatments. Larvae without deformities were selected and 10 larvae were transferred to 100 mL beakers filled with 80 mL of seawater. To test the effect of the salinity on larval survival, a range of salinity was established (2, 5, 10, 15, 20, 25, 30, 35) using 10 replicates per treatment (100 larvae per treatment; 800 larvae in total). This salinity range reflects salinities naturally experienced by adult populations (Mingkid et al., 2006b). Seawater was prepared by adding artificial seawater salts to distilled water. Salinity was confirmed measuring the specific gravity with a hydrometer, using the Practical Salinity Scale. To prevent excessive stress, larvae were acclimated to the final salinity conditions of each treatment by increasing or decreasing the salinity of broodstock system (Landschoff et al., 2013), five units every hour until reaching experimental conditions. According to this timeline, we expended 1 h to reach 30 and 20 of salinities, 2 h for 35 and 15 of salinity, 3 h for 10 of salinity 10, 4 h for 5 of salinity, and 4 h and 24 min for 2 of salinity (supplementary material 1). Once the larvae reach the targeted salinity level, we started the experiment at this level. The same acclimation protocol was used in the experiments 2 and 3. Culture beakers were incubated in a temperature-controlled room (20 °C and 12:12, L:D). To estimate the larval survival, the larvae were checked under the stereomicroscope and dead specimens were counted and removed at 2, 6, 12, and 24 h after starting the experiment. We decided that 24 h would be a realistic experiment duration to evaluate the short-term impact of the salinity, since modelling studies have shown that, under intermediate-strength tidal systems, larvae undergoing diel migration can be transported 4 km d^{-1} horizontally (Queiroga and Blanton, 2005). The magnitude of this transport would allow the larvae to move far enough from low salinity environments.

Larval survival rates were compared between salinity levels and time using a 2-way, permutational ANOVA test (Anderson, 2001), based on Euclidean distances calculated from square root-transformed data. The model included two factors treated as fixed: “Salinity” with seven levels (after removing salinity 2 due to no survival) and “Time” with four levels. P-values were calculated from 999 unrestricted permutations. Statistical analyses were carried out using the software PRIMER 7 & PERMANOVA+.

2.3. Experiment 2. passive sinking rate

The level of locomotory activity necessary to overcome the negative buoyancy was determined estimating the passive sinking rate at different salinity conditions (5, 10, 15, 20, 25, 30, 35). Seawater preparation and acclimation followed the same protocol as above. Following

Park et al. (2004) experiments were conducted in a clear plastic chamber measuring 5 × 5 × 30 cm in a temperature-controlled room (20 °C). After anaesthetized with ethyl carbamate (0.4 M for 5 min), individual larvae with the pleon folded ventrally were carefully introduced into the upper layer of the column using a pipette to let them sink passively. The first 15 cm of the chamber were used to achieve the terminal velocity, and then the time required for each larva to move the following 10 cm distance was measured to compute the sinking rate. A total of 20 larvae for each of the seven salinity levels were used to calculate a mean passive sinking rate. Larvae used for the experiment were derived from one single ovigerous crab. Kruskal-Wallis non-parametric test was applied to assess the difference in passive sinking rate between salinity levels.

2.4. Experiment 3. swimming patterns

In a temperature-controlled room (20 °C), the swimming behavior of *H. takanoi* larvae was recorded bi-dimensionally with a video camera (Sony, HDR-SR12, 30 frames s^{-1}) mounted in front of a 10 cm × 10 cm × 10 cm aquarium filled with 1 L of seawater. Because the behavior of decapod larvae is sensitive to light (Sulkin, 1984) the video recording was done in the dark using two infrared LED lamps (wavelength of 730 nm). Larvae used for the experiment were derived from one single ovigerous crab, that spawned two days after collection. For each salinity treatment (5, 10, 15, 20, 25, 30, 35 of salinity), 20 unfed zoea I larvae were pipetted from the acclimation vessels and were recorded. At a density of 0.02 larvae mL^{-1} the interactions between individuals were almost negligible and the overlap of swimming trajectories infrequent. Seawater preparation and acclimation of larvae follow the same protocol as in experiment 1. The observations were performed in absence and presence of food conditions (*Tetraselmis tetrathele*, prey density of 4000 cell mL^{-1}) for comparisons. It is true that salinity has an impact on the growth of *Tetraselmis* species, but it is well known their ability to tolerate a wide range of salt concentrations (Fabregas et al., 1984). Therefore, we assume that differences in salinity does not affect the phytoplankton distribution in the observational vessel.

After transferring the larvae into the aquarium, the behavior was recorded for 45 min, discarding the first 15 min to allow the turbulence to dissipate and larvae to adjust the experimental conditions. Then the larvae were transferred to a new aquarium with the same salinity but with food available to repeat the recording procedure. Therefore, each salinity level required 1.5 h of video recording (45 min without food and 45 min with food). To shorten the experiment duration, we used two video cameras in parallel to record the larval behavior in two salinity treatments at the same time. By doing this, the experiment was completed in 6 h (supplementary material 1). This procedure also minimizes the differences in starvation time between the larvae observed at the beginning (25 of salinity) and at the end of the experiment (5 and 10 of salinity).

To characterize the locomotion and trajectories, all recordings were reviewed by a video editing software (Grass Valley EDIUS), and a minimum of 50 trajectories were picked out for tracking, when possible. Coordinates for the gravity center of larvae were digitized in successive frames with the image processing software (Swallow Series image processing system, DigiMo) of Particle Tracking Velocimetry (PTV).

To calibrate the videos, we measured the number of pixels corresponding to 1 cm of a ruler immersed in the aquarium. Thus, pixel x-y co-ordinates were converted to physical positions and assembled into larval swimming trajectories by associating points in successive video frames that represented the same organism over time. The data obtained within 10 mm away from the aquarium wall were not used for analysis to prevent the wall effect.

For behavior analysis, we calculated the swimming direction and speed. The swimming direction was represented as the angle between two successive positions and the gravity direction. The instantaneous speed was estimated as the distance between two successive positions of a larvae divided by the time step of the camera (0.033 s). Because, the omission of the larval movement in the third dimension, we will use the term apparent swimming speed (Urbina et al., 2011). To visualize the difference in swimming speed between treatments, we used kernel density plots of instantaneous speed data performed on the whole set of trajectories (Michalec et al., 2010). Mean speeds were extracted for every trajectory available and used for statistical analysis. Difference in the apparent swimming speeds were compared between salinity levels, swimming direction and food conditions using a 3-way PERMANOVA test (Anderson, 2001), based on Euclidean distances calculated from square root-transformed data. The model included 3 factors treated as fixed: "Salinity" with 6 levels (after removing salinity 5 due to low number of trajectories extracted), "direction" with two levels (upward and downward swimming), and "Food" with two levels (absence and presence of food). Like in previous analysis, *p*-values were calculated from 999 unrestricted permutations of the raw data, and statistical analyses were carried out using the software PRIMER 7 & PERMANOVA+. Plots were performed using the statistical computing software R with the RStudio interface (version 3.3.3; RStudio Team, 2016) with added functionality from the associated packages "ggplot 2" (Wickham, 2016) and "openair" (Carslaw and Ropkins, 2012).

3. Results

3.1. Larval survival

Salinity showed a positive effect on the larval survival, with highest survival rates at salinity of 25, 30, 35 (Fig. 1). This difference on the larval survival for the factor "salinities" was also detected by the PERMANOVA test (Pseudo-F = 2.79, p = 0.002). On the contrary, low salinity of 2 and 5 were lethal after 2 h and 12 h of incubation respectively. Larvae showed survival rates higher than 50% from salinities of 10 during the first 2 h, but the survival dropped significantly at 6 h of experiment for the salinities of 10, 15, and 20. Despite the higher mortality during the first 6 h of experiment, PERMANOVA did not detect significant effect of the factor "time" on the larval survival (Pseudo-F = 1.20, p = 0.254) (Fig. s1).

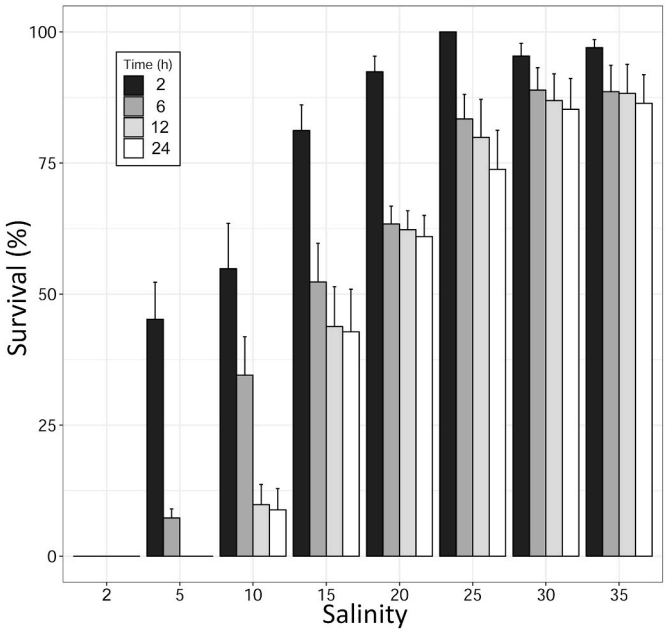

Fig. 1. Survival rate of newly hatched *Hemigrapsus takanoi* larvae (mean ± SD) at different incubation times and salinities.

3.2. Passive sinking rate

The larvae showed negative buoyancy and the sinking rate increased significantly as the salinity decreased (KW test, $p < 0.001$). Thus, the mean sinking rate decreased from 3.55 ± 0.33 mm s^{-1} at salinity of 5 to a minimum of 2.36 ± 0.16 mm s^{-1} at salinity of 35 (Table 1).

3.3. Swimming patterns

Larvae of *H. takanoi* displayed complex and variable swimming patterns. Individual swimming trajectories visually varied in terms of speed and direction of the motion (Fig. 2). Thus, at salinities lower than 20 the larvae displayed slower apparent swimming speeds than at higher salinities. In absence of food, larvae showed more variable swimming patterns, displaying spiral movements and changes in the trajectory more frequently, denoting a cruising behavior. When the food was offered, swimming trajectories became straighter and more vertical. This pattern was clear at salinities higher than 20, whereas at salinities of 15 and 10 the effect of food availability seemed to disappear (Fig. 2).

The PERMANOVA test detected significant differences in mean instantaneous apparent swimming speed between the six salinity conditions (Pseudo-$F = 2.346$, $p = 0.025$), food condition (Pseudo-$F = 6.922$, $p = 0.010$) and jump direction (Pseudo-$F = 7.756$, $p = 0.008$), whereas the interaction was significant only between salinity and food (Pseudo-$F = 1.908$, $p = 0.024$) (Table 2).

Maximum instantaneous speed (10.12 mm s^{-1}) was recorded at salinity of 30 without food, but most of the speed values were smaller than 5 mm s^{-1} (Fig. 3). Upward trajectories showed higher mean instantaneous apparent swimming speeds for all salinities and food treatments (only salinity of 10 and no food showed opposite pattern) (Table 1), and the mean speed was higher when the food was available. For both food treatments the mean instantaneous apparent swimming speed of the upward movements increased from salinity of 10–35. Thus, in absence of food, the increase was from 1.21 ± 0.27 mm s^{-1} to 2.09 ± 0.49 mm s^{-1}, whereas under food conditions it was from 2.20 ± 0.23 mm s^{-1} to 3.73 ± 1.12 mm s^{-1} (Table 1).

Density plots in Fig. 3 showed the same observed patterns comparing the mean instantaneous apparent swimming speed values, but also showed interesting information on the distribution of the speed data. For instance, the density plots displayed a right skewed distribution of the data towards the higher instantaneous apparent swimming speed values that implies these high values are not frequent. In addition, the density plots exhibited a higher frequency of lower instantaneous apparent swimming speed values in the downward trajectories, and that it was more evident at salinities higher than 20.

In Fig. 4, the polar plots show the frequency of trajectories by swimming direction and speed. Under food conditions, the swimming trajectories were dominated by vertical directions (near 90° and 270°) and higher frequency of upward movements, especially at salinities higher than 20. At lower salinities, the pattern tended to disappear, and the larvae displayed a more diverse trajectories direction and relatively lower apparent swimming speeds. When the food was not available, the larvae showed a more random swimming trajectories, similar to the behavior displayed with food and at low salinity conditions. At salinity of 10, larvae showed a higher frequency of vertical movements, but although we should note that in this case, it is mostly due to short upward motion that ended up sinking to the bottom of the aquarium.

4. Discussion

The dispersal of *H. takanoi* in the non-native populations of Europe depends on the capacity of the early stage zoeae to overcome environmental stressors that can compromise their performance or even survival during the free-living planktonic phase. Here we have shown that newly hatched *H. takanoi* zoeae cannot tolerate a wide range of salinities, and the residence time at certain salinity levels has an impact on the survival. Only at salinities higher than 20, the 50% of the larvae can survive 24 h of incubation. These results are consistent with previous laboratory experiments (Mingkid et al., 2006a), which indicated that early larval stages of *H. takanoi* were stenohaline and required to perform an export strategy to find higher salinity conditions to complete the development. Moreover, we found poor tolerance to low salinity conditions even at short-time exposure. Therefore, this suggests that newly hatched larvae need to be transported offshore quickly, especially for those larvae produced at low salinity locations, since staying there just for a few hours can be lethal.

Swimming performance is key to complete selective tidal-stream transport towards higher salinity environments (López-Duarte and Tankersley, 2007). In general, zoeae of brachyuran crabs are relatively strong swimmers and, by either maxillipeds oscillations or by abdominal contractions, they are capable of sustaining speeds on the order of 5–15 mm s^{-1} (Sulkin, 1984; Ford et al., 2005). *Hemigrapsus takanoi* exhibited variable vertical swimming speeds but normally around 2–5 mm s^{-1}, and lower than 10 mm s^{-1}, which are similar to those observed in *H. sanguineus* (Park et al., 2004). At this range of apparent swimming speeds, upward vertical displacements should allow the larvae to reach the surface of the water column and facilitates export from estuaries (Epifanio and Cohen, 2016). Moreover, upward trajectories are faster than downward ones, and the passive sinking speeds recorded using anaesthetized larvae were always faster than those observed with active larvae for each salinity range, indicating a certain level of motility exists during the downward movement. This behavioral feature may be related with the negative geotaxis and barokinesis response that facilitates *H. sanguineus* and *H. oregonensis* stage I zoea to perform the mentioned selective tidal-stream transport (Arana and Sulkin, 1993; Park et al., 2004). We have to point out that we calculated the swimming speed collapsing the 3-dimensional trajectories into 2-dimensional projections. It is well documented that, while estimations derived from 2-dimensional observations represents the true value of the passive sinking speed, swimming speeds of real cruising trajectories are underestimated (Kiørboe and Bagøien, 2005; Urbina et al., 2011). Bias in 2-dimensional observations is frequent in behavioral studies of

Table 1
Passive sinking rate and mean instantaneous swimming speeds (mm s^{-1} ± SD) at different salinity conditions and presence/absence of food. The mean instantaneous swimming speeds for each food treatment is shown for the upward and downward movements.

Salinity	Passive sinking	Food		No food	
		up	down	up	down
5	3.55 ± 0.33	–	–	–	–Urbina et al., 2011
10	3.34 ± 0.23	2.20 ± 0.23	2.00 ± 0.55	1.21 ± 0.27	1.36 ± 0.32
15	3.13 ± 0.33	2.12 ± 0.57	1.69 ± 0.42	1.91 ± 0.75	1.40 ± 0.40
20	2.72 ± 0.12	2.32 ± 0.56	1.94 ± 0.45	1.97 ± 0.74	1.50 ± 0.50
25	2.57 ± 0.07	2.98 ± 0.62	1.61 ± 0.43	2.46 ± 0.95	1.73 ± 0.55
30	2.48 ± 0.09	3.54 ± 1.15	2.29 ± 0.82	2.57 ± 0.89	1.66 ± 0.48
35	2.36 ± 0.16	3.73 ± 1.12	2.19 ± 0.81	2.09 ± 0.49	1.57 ± 0.30

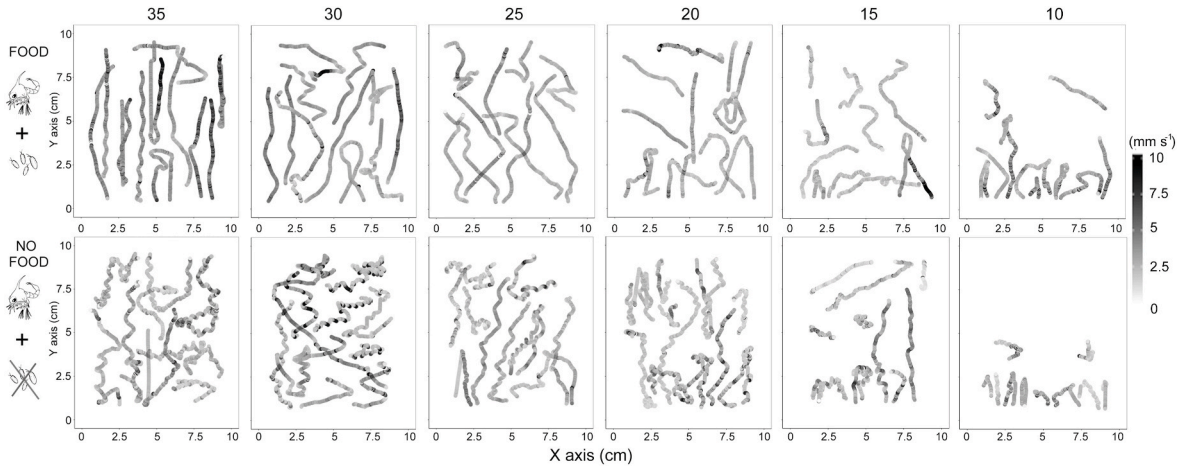

Fig. 2. Representative larval swimming trajectories of *Hemigrapsus takanoi* larvae under different levels of salinity and food availability extracted from video clips. The instantaneous swimming speed (mm s^{-1}) of each frame is represented in the greyscale.

Table 2
Results of three-way PERMANOVA testing for effects of salinity, food and swimming direction on the swimming speed of *Hemigrapsus takanoi* larvae. Significant *p* values in bold.

Source of variation	df	SS	MS	Pseudo-*F*	*p* (perm)
Salinity	5	16.057	3.211	2.346	**0.025**
Food	1	9.476	9.476	6.922	**0.010**
Direction (up/down)	1	10.617	10.617	7.756	**0.008**
Salinity x Food	5	13.057	2.611	1.908	**0.024**
Salinity x Direction	5	8.625	1.725	1.260	0.234
Food x Direction	1	1.466	1.466	1.070	0.419
Residual	5	6.844	1.369		
Total	23	66.142			

invertebrate larvae (i.e. Chan et al., 2015; Sorochan and Metaxas, 2017; Maciejewski et al., 2019). In fact, to our knowledge, all data available in the literature for decapod larvae are based on 2-dimensional studies making them comparable with our apparent swimming speed results (e.g. Sulkin, 1984; Arana and Sulkin, 1993; Park et al., 2004; Ford et al., 2005; Caracappa and Munroe, 2019).

In the present study, the swimming behavior of *H. takanoi* larvae varied depending on the salinity conditions. Broadly, we observed lower swimming performance at salinities lower than 20. At these conditions, larvae displayed slower swimming, as well as shorter and more random trajectories. High salinity aids the larvae to sink slower and can require using less energy to swim. Thus, suboptimal salinity conditions over extended periods could result in an increased energy expenditure to maintain a zoea's vertical position (Caracappa and Munroe, 2018). It is true that since female brachyuran crabs attach their embryos externally, it may allow them to acclimate to low salinities during embryogenesis and potentially increasing their ability to tolerate low salinities after hatching in *Hemigrapsus* species (Giménez and Torres, 2002; Taylor and Seneviratna, 2005). In our experiment, we collected the ovigerous crabs from the same locality and incubated them in the laboratory at a salinity of the field condition. It makes sense that in the field, larvae produced from lower salinities could show higher survival and swimming performance than those observed in our experiments. However, Urzúa et al. (2018) found that, in *H. crenulatus*, the embryogenesis is not completed at low salinities of 5 because the female cannot save energy of osmoregulation process to invest enough energy in the egg yolk. In addition, these authors also found that larvae hatched from eggs incubated at high salinity of 30 were larger in size, had higher dry weight, carbon content and energy than larvae hatched from eggs produced at salinity of 15 (Urzúa et al., 2018). It suggests that the observed decrease in the apparent swimming speed and activity with decreasing salinity may be related to the drop in energy allocated to swimming. Similar salinity-related constraints in the swimming performance have been reported in copepods (Michalec et al., 2010). Therefore, crab populations located at lower salinity condition may produce lower quality of larvae, that likely perform sub-optimal swimming behavior and survival rates, as we observed in the present study.

The experiments performed in this study required an acclimation

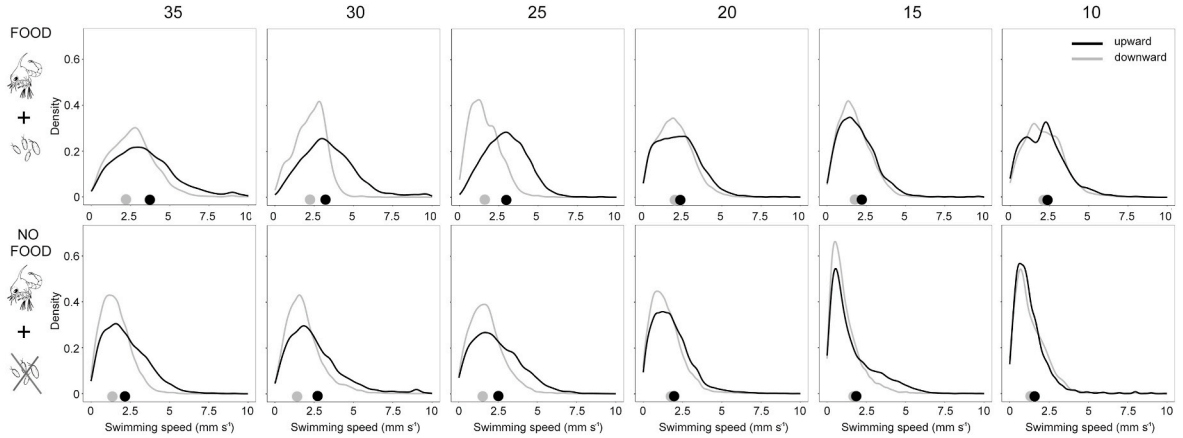

Fig. 3. Kernel density plots of instantaneous swimming speed at different levels of salinity and food availability extracted from video clips. The data extracted from upward and downwards trajectories are highlighted in black and grey, respectively. The mean swimming speed is denoted by black and grey circles for reference.

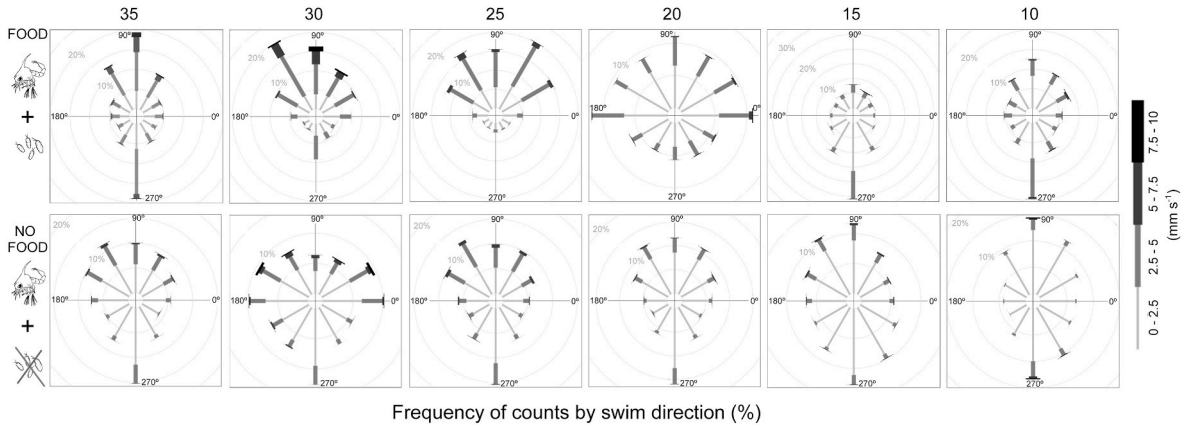

Fig. 4. WindRose function to plot swimming speed/direction frequencies for each salinity level and food condition. Swimming speeds are split into four intervals shown by the scale in the panel. The grey circles show the % frequencies increasing every 5%.

procedure before starting the observations. Therefore, it is reasonable to think that larvae would have had different energy reserves at the end of the experiment due to starvation, and that can affect the swimming behavior or survival. Severe food limitations cause losses of biomass and energy, delay of the molt cycle, and lower survival rates of planktotrophic larvae (Guerao et al., 2012). Under stressful environmental conditions, like low salinity, the effect of starvation can be even more acute. In this sense, it is possible that in experiment 1, after 24 h of starvation larval mortality can be higher at low salinity. We observed that the mortality rate actually decreased from one checkpoint time to the following one. Thus, if we estimate the mortality rate per hour, the rate is higher from 6 to 12 h than from 12 to 24 h at stressful conditions of low salinity (e.g., 10, 15). The drop in mortality rate through the experiment suggest that the starvation cannot explain the mortality.

In relation to the experiment 3, it is not well studied the influence of short starvation periods (6 h in our study) since the effects of food limitation are mostly evaluated at a scale of one or a few days by means of 'point of-no-return' (Anger, 2001). In this sense, we cannot rule out that the short-term starvation used to perform the experiment 3 affects the larval behavior. Still differences in swimming performance of larvae recorded at different salinity conditions and the same starvation time, indicate that food limitation has no evident effect. For instance, the salinity treatments of 35 and 15 were recorded at the same time, after 4.5 h of starvation (Supplementary material 1), however the larvae at 35 of salinity performed faster, longer and more vertical trajectories than the larvae swimming in the salinity of 15. Therefore, we assume that this level of starvation has limited impact on the interpretation of the swimming behavior results.

We also found differences in the swimming behavior related with food availability, especially at salinities higher than 20. In this sense, under food conditions the vertical, upward trajectories were more frequent, whereas the angle of the swimming trajectories was more random and erratic when the food was not available. Interestingly, without food, the larvae tend to swim slower and in helical trajectories more frequently. Helical swimming behavior is well documented in invertebrate larvae across many taxa; however, the function of this pattern remains unknown (Chia et al., 1984). One potential benefit of helical swimming is to increase the time a larva spends in a particular stratum, allowing the larvae to scan the environment for cues (Chan, 2012). There are evidences in the literature suggesting that increased helical swimming behavior increases food capture in protists (Menden-Deuer and Grünbaum, 2006) and copepods (Caparroy et al., 1998). Recent study on the swimming behavior of oyster larvae has shown that decreasing settlement cues the proportion of larvae swimming in helices increased; however, larvae did not increase their helical swimming in response to food availability, which suggested that helices may be an exploratory behavior (Maciejewski et al., 2019). Our observations in crab zoea also indicate that they use helical trajectories to explore the water to search for environmental cues. Thus, the function of helical swimming behavior varies across taxonomic groups.

In conclusion, using video-tracking techniques, we have shown that salinity can modify the swimming behavior of newly hatched *H. takanoi* larvae. In this sense, the apparent swimming velocity and angle trajectory indicate suboptimal conditions at salinities lower than 20. Consequently, larvae produced at low salinity habitats would display a lower swimming performance that could compromise the behavioral mechanisms involved in the offshore transport towards more salty waters, where the larva could develop well. The predicted lower larval survival at low salinity habitat conditions implies not only a lower dispersal capacity locally, but also a limited invasiveness. Therefore, the quantitative information on larval swimming behaviors and complex responses to environmental conditions can be implemented in biophysically coupled larval dispersal models, useful for the monitoring of this invasive species in the European coast.

Credit author statement

Jose M. Landeira: Conceptualization, Investigation, Data curation, Writing- Original draft preparation; **Baobo Liu**: Data curation, Formal analysis, Investigation; **Takuo Omura**: Methodology, resources; **Tatsuro Akiba**: Methodology, Formal analysis; **Yuji Tanaka**: Supervision, Funding acquisition, Writing - Review & Editing.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecss.2020.106976.

References

Anderson, M.J., 2001. Permutational tests for univariate or multivariate analysis of variance and regression. Can. J. Fish. Aquat. Sci. 58, 626–639.

Anger, K., 2001. The Biology of Decapod Crustacean Larvae. A.A. Balkema, Lisse.

Anger, K., 2003. Salinity as a key parameter in the larval biology of decapod crustaceans. Invertebr. Reprod. Dev. 43, 29–45. https://doi.org/10.1080/07924259.2003.9652520.

Arana, M., Sulkin, S., 1993. Behavioral basis of depth regulation in the first zoeal stage of the Pacific shore crab, *Hemigrapsus oregonensis* (Brachyura: Grapsidae). Pac. Sci. 47, 256–262.

Asakura, A., Watanabe, S., 2005. *Hemigrapsus takanoi*, new species, a sibling species of the common Japanese intertidal crab *H. penicillatus* (Decapoda: brachuyra: Grapsoidea). J. Crustac Biol. 25, 279–292.

Ashelby, C.W., Sewell, J., Rostron, J., Shrubsole, R., Child, T., Clark, P.F., 2017. Evidence for the invasion and successful establishment of *Hemigrapsus takanoi* Asakura & Watanabe, 2005 (Crustacea: Decapoda: Varunidae) in great Britain. Crustaceana 90, 695–708.

Caparroy, P., Pérez, M.T., Carlotti, F., 1998. Feeding behaviour of *Centropages typicus* in calm and turbulent conditions. Mar. Ecol. Prog. Ser. 168, 109–118. https://doi.org/10.3354/meps168109.

Caracappa, J.C., Munroe, D.M., 2018. Morphological variability among broods of first-stage blue crab (*Callinectes sapidus*) zoeae. Biol. Bull. 235, 123–133. https://doi.org/10.1086/699922.

Caracappa, J.C., Munroe, D.M., 2019. Variability in swimming behavior among broods of blue crab (*Callinectes sapidus*) zoeae. J. Exp. Mar. Biol. Ecol. 518, 151176 https://doi.org/10.1016/j.jembe.2019.151176.

Carslaw, D.C., Ropkins, K., 2012. Openair — an R package for air quality data analysis. Environ. Model. Software 27–28, 52–61. https://doi.org/10.1016/j.envsoft.2011.09.008.

Chan, K.Y.K., 2012. Biomechanics of larval morphology affect swimming: insights from the sand dollars *Dendraster excentricus*. Integr. Comp. Biol. 52, 458–469. https://doi.org/10.1093/icb/ics092.

Chan, K.Y.K., García, E., Dupont, S., 2015. Acidification reduced growth rate but not swimming speed of larval sea urchins. Sci. Rep. 5, 9764. https://doi.org/10.1038/srep09764.

Chia, F.-S., Buckland-Nicks, J., Young, C.M., 1984. Locomotion of marine invertebrate larvae: a review. Can. J. Zool. 62, 1205–1222. https://doi.org/10.1139/z84-176.

Cowen, R.K., Sponaugle, S., 2009. Larval dispersal and marine population connectivity. Annu. Rev. Mar. Sci. 1, 443–466. https://doi.org/10.1146/annurev.marine.010908.163757.

Diele, K., Simith, D.J.B., 2006. Salinity tolerance of northern Brazilian mangrove crab larvae, *Ucides cordatus* (Ocypodidae): necessity for larval export? Estuar. Coast Shelf Sci. 68, 600–608. https://doi.org/10.1016/j.ecss.2006.03.012.

Epifanio, C.E., Cohen, J.H., 2016. Behavioral adaptations in larvae of brachyuran crabs: a review. J. Exp. Mar. Biol. Ecol. 482, 85–105. https://doi.org/10.1016/j.jembe.2016.05.006.

Evans, D.H., 2008. Osmotic and Ionic Regulation: Cells and Animals. CRC Press, Boca Raton, FL. USA.

Fabregas, J., Abalde, J., Herrero, C., Cabezas, B., Veiga, M., 1984. Growth of the marine microalga *Tetraselmis suecica* in batch cultures with different salinities and nutrient concentrations. Aquaculture 42, 207–215. https://doi.org/10.1016/0044-8486(84)90101-7.

Ford, M.D., Schuegraf, M.J., Elkink, M.S., Edwin DeMont, M., 2005. A comparison of swimming structures and kinematics in three species of crustacean larvae. Mar. Freshw. Behav. Physiol. 38, 79–94. https://doi:10.1080/10236240500148934.

Giménez, L., Torres, G., 2002. Larval growth in the estuarine crab *Chasmagnathus granulata*: the importance of salinity experienced during embryonic development, and the initial larval biomass. Mar. Biol. 141, 877–885. https://doi.org/10.1007/s00227-002-0887-5.

Guerao, G., Simeó, C.G., Anger, K., Urzúa, Á., Rotllant, G., 2012. Nutritional vulnerability of early zoea larvae of the crab *Maja brachydactyla* (Brachyura, Majidae). Aquat. Biol. 16, 253–264. https://doi.org/10.3354/ab00457.

Hudson, D.M., Sexton, D.J., Wint, D., Capizzano, C., Crivello, J.F., 2018. Physiological and behavioral response of the Asian shore crab, *Hemigrapsus sanguineus*, to salinity: implications for estuarine distribution and invasion. PeerJ 6, e5446. https://doi.org/10.7717/peerj.5446.

Kiørboe, T., Bagøien, E., 2005. Motility patterns and mate encounter rates in planktonic copepods. Limnol. Oceanogr. 50, 1999–2007. https://doi.org/10.4319/lo.2005.50.6.1999.

Landeira, J.M., Cuesta, J.A., Tanaka, Y., 2019. Larval development of the brush-clawed shore crab *Hemigrapsus takanoi* Asakura & Watanabe, 2005 (Decapoda, Brachyura, Varunidae). J. Mar. Biol. Assoc. U. K. 99, 1153–1164. https://doi.org/10.1017/S002531541900002X.

Landschoff, J., Lackschewitz, D., Kesy, K., Reise, K., 2013. Globalization pressure and habitat change: Pacific rocky shore crabs invade armored shorelines in the Atlantic Wadden Sea. Aquat. Invasions 8, 77–87. https://doi.org/10.3391/ai.2013.8.1.09.

López-Duarte, P.C., Tankersley, R.A., 2007. Circatidal swimming behavior of brachyuran crab zoea larvae: implications for ebb-tide transport. Mar. Biol. 151, 2037–2051. https://doi.org/10.1007/s00227-007-0614-3.

Maciejewski, M.F., Meyer, K.S., Wheeler, J.D., Anderson, E.J., Pittoors, N.C., Mullineaux, L.S., 2019. Helical swimming as an exploratory behavior in competent larvae of the eastern oyster (*Crassostrea virginica*). J. Exp. Mar. Biol. Ecol. 510, 86–94. https://doi.org/10.1016/j.jembe.2018.10.007.

Makino, W., Miura, O., Kaiser, F., Geffray, M., Katsube, T., Urabe, J., 2018. Evidence of multiple introductions and genetic admixture of the Asian brush-clawed shore crab *Hemigrapsus takanoi* (Decapoda: Brachyura: Varunidae) along the Northern European coast. Biol. Invasions 20, 825–842. https://doi.org/10.1007/s10530-017-1604-0.

Menden-Deuer, S.M., Grünbaum, D., 2006. Individual foraging behaviors and population distributions of a planktonic predator aggregating to phytoplankton thin layers. Limnol. Oceanogr. 51, 109–116.

Michalec, F.G., Souissi, S., Dur, G., Mahjoub, M.S., Schmitt, F.G., Hwang, J.S., 2010. Differences in behavioral responses of *Eurytemora affinis* (Copepoda, Calanoida) reproductive stages to salinity variations. J. Plankton Res. 32, 805–813. https://doi.org/10.1093/plankt/fbq006.

Mingkid, W.M., Yokota, M., Watanabe, S., 2006a. Salinity tolerance of larvae in the Penicillate crab *Hemigrapsus takanoi* (Decapoda: Brachyura: Grapsidae). La mer 44, 17–21.

Mingkid, W.M., Akiwa, S., Watanabe, S., 2006b. Morphological characteristics, pigmentation, and distribution of the sibling penicillate crabs, *Hemigrapsus penicillatus* (de haan, 1835) and *H. takanoi* Asakura & Watanabe, 2005 (Decapoda, Brachyura, Grapsidae) in Tokyo bay. Crustaceana 79, 1107–1121. https://doi.org/10.1163/156854006778859696.

Noël, P., Tardy, E., d'Udekem d'Acoz, C., 1997. Will the crab *Hemigrapsus penicillatus* invade the coasts of Europe? C. R. Acad. Sci. III-Vie 320, 741–745.

Park, S., Epifanio, C.E., Grey, E.K., 2004. Behavior of larval *Hemigrapsus sanguineus* (de Haan) in response to gravity and pressure. J. Exp. Mar. Biol. Ecol. 307, 197–206. https://doi.org/10.1016/j.jembe.2004.02.007.

Queiroga, H., Blanton, J., 2005. Interactions between behavior and physical forcing in the control of horizontal transport of decapod crustacean larvae. Adv. Mar. Biol. 47, 107–213.

Shinji, J., Strüssmann, C.A., Wilder, M.N., Watanabe, S., 2009. Short-term responses of the adults of the common Japanese intertidal crab, *Hemigrapsus takanoi* (Decapoda: Brachyura: grapsoidea) at different salinities: osmoregulation, oxygen consumption, and ammonia excretion. J. Crustac Biol. 29, 269–272. https://doi.org/10.1651/08-2998.1.

Sorochan, K.A., Metaxas, A., 2017. The effect of temperature on motility of the nauplius and cypris stages of the acorn barnacle *Semibalanus balanoides*. Mar. Ecol. Prog. Ser. 579, 55–66. https://doi.org/10.3354/meps12246.

Sulkin, S., 1984. Behavioral basis of depth regulation in the larvae of brachyuran crabs. Mar. Ecol. Prog. Ser. 15, 181–205. https://doi.org/10.3354/meps015181.

Taylor, H.H., Seneviratna, D., 2005. Ontogeny of salinity tolerance and hyper-osmoregulation by embryos of the intertidal crabs *Hemigrapsus edwardsii* and *Hemigrapsus crenulatus* (Decapoda, Grapsidae): survival of acute hyposaline exposure. Comp. Biochem. Physiol. - A Mol. Integr. Physiol. 140, 495–505. https://doi.org/10.1016/j.cbpb.2005.03.005.

RStudio Team, 2016. RStudio. Integrated Development for R. RStudio, Inc., Boston, MA. http://www.rstudio.com/.

Urbina, M., Forster, M., Glover, C., 2011. Leap of faith: Voluntary emersion behaviour and physiological adaptations to aerial exposure in a non-aestivating freshwater fish in response to aquatic hypoxia. Physiol. Behav. 103 (2), 240–247. https://doi.org/10.1016/j.physbeh.2011.02.009.

Urbina, M., Paschke, K., Gebauer, P., Chaparro, O.R., 2010. Physiological energetics of the estuarine crab *Hemigrapsus crenulatus* (Crustacea: Decapoda: Varunidae): responses to different salinity levels. J. Mar. Biol. Assoc. U. K. 90, 267–273. https://doi:10.1017/S0025315409990889.

Urzúa, Á., Urbina, M.A., 2017. Ecophysiological adaptations to variable salinity environments in the crab *Hemigrapsus crenulatus* from the Southeastern Pacific coast: sodium regulation, respiration and excretion. Comp. Biochem. Physiol. Part A Mol. Integr. Physiol. 210, 35–43. https://doi.org/10.1016/j.cbpa.2017.05.010.

Urzúa, Á., Bascur, M., Guzmán, F., Urbina, M., 2018. Carry-over effects modulated by salinity during the early ontogeny of the euryhaline crab *Hemigrapsus crenulatus* from the Southeastern Pacific coast: development time and carbon and energy content of offspring. Comp. Biochem. Physiol. Part A Mol. Integr. Physiol 217, 55–62. https://doi.org/10.1016/j.cbpa.2018.01.001.

Wickham, H., 2016. Ggplot2: Elegant Graphics for Data Analysis. Springer, New York.