Ontogeny of osmoregulation of the Asian shore crab *Hemigrapsus sanguineus* at an invaded site of Europe

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We studied the ontogeny of osmoregulation of the Asian shore crab *Hemigrapsus sanguineus* at an invaded area in the North Sea. *H. sanguineus* is native to Japan and China but has successfully invaded the Atlantic coast of North America and Europe. In the invaded areas, *H. sanguineus* is becoming a keystone species as driver of community structure and the adults compete with the shore crab *Carcinus maenas*. Strong osmoregulatory abilities may confer the potential to use and invade coastal areas already earlier in the life cycle. We reared larvae and first juveniles at 24 °C in seawater from hatching to intermoult of each developmental stage (zoea I-V, megalopa, crab I). We exposed each stage to a range of salinities (0–39 ppt) for 24 h, and then we quantified haemolymph osmolality, using nano-osmometry. In addition, we quantified osmolality in field-collected adults after acclimation to the test salinities for 6 days. Larvae of *H. sanguineus* were able to hyper-osmoregulate at low salinities (15 and 20 ppt) over the complete larval development, although the capacity was reduced at the zoeal stage V; at higher salinities (25–39 ppt), all larval stages were osmoconformers. The capacity to slightly hypo-regulate at high salinity appeared in the first juvenile. Adults were able to hyper-osmoregulate at low salinities and hypo-regulate at concentrated seawater (39 ppt). *H. sanguineus* showed a strong capacity to osmoregulate as compared to its native competitor *C. maenas*, which only hyper-regulates at the first and last larval stages and does not hypo-regulate at the juvenile-adult stages. The capacity of *H. sanguineus* to osmoregulate over most of the life cycle should underpin the potential to invade empty niches in the coastal zone (characterized by low salinity and high temperatures). Osmoregulation abilities over the whole life cycle also constitute a strong competitive advantage over *C. maenas*.

**Key words:** shore crab, osmoregulation, ontogeny, larva, Invasive species

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**Introduction**

Understanding and predicting the process of establishment of exotic species require better knowledge of the traits promoting invasion and colonization of new habitats. Physiological traits, as the foundation of environmental tolerance, are central to the process of invasion and species distribution (Ames et al., 2020; Kelley, 2014). The capacity to osmoregulate is a key adaptation for the use of coastal and estuarine habitats (Péqueux, 1995; Charmantier, 1998; Anger, 2003). Osmoregulation (here referring to extracellular osmoregulation) is the capacity of an organism to actively regulate the osmotic pressure of the body fluids (Péqueux, 1995; Charmantier et al., 2009; Evans and Claiborne, 2009; Lignot and Charmantier, 2015; Rahi et al., 2018). Osmoregulation enables organisms to achieve optimal functioning over a range of salinities, maintain the concentration of essential substances, keep acid–base balance for the proper cell function (Whiteley, 2011; Henry et al., 2012; Whiteley and Taylor, 2015) and sustain growth and development (Torres et al., 2011). Osmoregulation is also likely to confer some capacity to tolerate ocean acidification conditions (Whiteley, 2011; Whiteley et al., 2018) and to facilitate evolutionary transitions from the marine to semi-terrestrial habitats (Anger and Charmantier, 2000).

Crustaceans are one of the most important groups of invaders in coastal areas (Galil et al., 2011). Tolerance to low salinity is a key trait within the most notable crustacean invaders (Rudnick et al., 2005; Roche et al., 2009; Fowler et al., 2013); low, high or variable salinities are characteristic of intertidal zones and estuaries. In invasive crustaceans, the ontogeny of osmoregulation is likely to influence the process of invasion through changes in the so-called ‘propagule pressure’ (sensu Simberloff, 2009). Little propagule pressure towards coastal-estuarine habitats would be expected from marine crustaceans, as they generally are stenohaline and weak osmoregulators or osmoconformers over their full life cycle (i.e. the osmolality of their body fluids is roughly equal to that of the surrounding fluid: Péqueux, 1995; Charmantier et al., 2009). By contrast, invasive estuarine species are osmoregulators at least during the adult stage (Rudnick et al., 2005; Fowler et al., 2013), but the full process of invasion may also depend on the life-history strategy during the larval phase. Estuarine species or those living in land-locked habitats show a pattern of osmoregulation that varies according to the strategy of ontogenetic migration. For instance, species retaining their larvae in the parental habitat (e.g. within estuaries or other land-locked water masses) are able to osmoregulate over the full life cycle (Charmantier et al., 1998; Anger and Charmantier, 2000, 2011). Therefore, for an invasive species following a larval retention strategy, invasion could theoretically take place at any stage of development. By contrast, the process of invasion will be restricted to specific life stages in species following the larval export strategy, i.e. where larvae are exported to coastal waters, characterized by higher and more stable salinities. In such species, the osmoregulatory capacity (OC), and hence the ability to use estuarine waters as habitat, is reduced or absent during the osmoconforming larval stages (Charmantier et al., 2002; Gieluch et al., 2004; Anger et al., 2008).

The ontogeny of osmoregulation should also drive the capacity of an exotic species to establish a self-sustaining population in a new habitat. Self-sustaining populations are a key for the long-term establishment, as well as for range expansion, a major characteristic of invasions (Gurevitch et al., 2011). Theoretically, self-sustaining populations can play a role in sustaining sink populations at the range limit (Dauphinais et al., 2018; Giménez et al., 2020). In species showing larval export strategy, successful establishment of local populations must rely in the capacity of larvae to perform ontogenetic migrations from and to the parental habitats. However, such capacity can vary regionally depending on hydrodynamic processes driving larval transport (Schab et al., 2013; Shanks et al., 2017), as well as the timing of reacquisition of the capacity to osmoregulate (Torres et al., 2006). By contrast, in species that are able to osmoregulate over the full life cycle, the establishment of local self-sustaining populations should not be restricted to specific life phases.

Here we report on the ontogeny of osmoregulation of the Asian shore crab *Hemigrapsus sanguineus*. *H. sanguineus* is native to the Pacific coast (Japan, China and Russia; Stephenson et al., 2009), but over the past 50 years, it has invaded the Atlantic coasts of North America and North Europe. In North America, *H. sanguineus* was first recorded in the 1980s, in the Delaware Bay and it subsequently expanded northwards over 10 degrees of latitude (Epifanio et al., 1998; Stephenson et al., 2009; Epifanio, 2013; Lord and Williams, 2017). In Europe, *H. sanguineus* was first found in the Dutch delta system in 1999 (Dauvin et al., 2009) and then it expanded over the North Sea (Jungblut et al., 2017; Geburzi et al., 2018; Jungblut et al., 2018), reaching also the coast of Scandia-

vania (Karlsson et al., 2019). In the German Bight (North Sea), *H. sanguineus* has invaded the intertidal zones of the Wadden Sea where densities average reached values of the order of 500 crabs m$^{-2}$ (Geburzi et al., 2018; Fig 3, C6: size range ≤ 10 mm).

The impact of *H. sanguineus* on other species, including mussels and crabs, has been recorded in both the Atlantic coast of USA and in N. Europe (Stephenson et al., 2009). As a mode of comparison, in the Atlantic coast of USA, the increase in abundance of *H. sanguineus* correlates with the disappearance of another invasive species, the shore crab *Carcinus maenas* (Lohrer and Whitlatch, 2002), which has invaded coastal worldwide (Carlton and Cohen, 2003; Roman and Palumbi, 2004) and is included in the list of the top 100 alien invasive species in the world (Global Invasive Species Database, 2021). In the US coast, adults of *H. sanguineus* outcompete *C. maenas* through predation on juveniles and shifts in diet that ultimately drive fecundity (Jensen et al., 2002; Griffen et al., 2011). We know less about how those species compare at their dispersive larval phase, a key trait driving invasions (Hassall et al., 2008; Simberloff, 2009).
**Materials and Methods**

**Collection of adults and larval rearing of H. sanguineus (De Haan, 1835)**

Experiments were carried out with adults and larvae from the local population of Helgoland and Sylt (North Sea, German Bight) during the reproductive period. Egg-carrying females were collected in intertidal habitats and kept in the Helgoland laboratory in 2 l. aquaria with oxygenated and filtered (0.2 μm) natural seawater (32.5 ppt). Aquaria were placed in a temperature-controlled room at 24°C with a 12:12 h light:dark cycle.

Experiments with adults were based on field-collected individuals. Experiments with larvae and first stage juveniles were run using standard methods of larval rearing (Charmantier et al., 2002; Torres et al., 2020, 2021b). Larvae were reared in filtered aerated natural seawater in groups of 50 individuals in 500 ml glass bowls (density: 0.1 larva ml⁻¹) in a temperature-controlled room at 24°C, 12:12 h light:dark cycle. Daily, rearing bowls were rinsed and cleaned, water was changed, dead individuals were removed and larvae were fed with freshly hatched Artemia sp. nauplii. Prior to experiments, larvae moulting to each stage were separated daily from the cultures and kept in additional bowls in order to obtain larvae with the same moulting age. Survival was tested at each stage at all salinities. Animals were checked at regular intervals and determined as dead when not moving after being repeatedly touched with a probe.

**Experiments**

Osmolality was adjusted with a salinometer (Cond 3110 SET1, WTW GmbH, Weilheim, Germany) by diluting natural seawater (32.5 ppt = 966 ± 1 mOsm kg⁻¹) with appropriate amounts of tap water. Water (samples of 20 μl) was then checked to achieve the appropriate osmolality using a micro-osmometer (Model 3MO, Advanced Instruments, Needham Heights, MA, USA); conversion factors between osmolality and salinity are as follows: 3.36 ppt (2021) = 100 mOsm kg⁻¹; 29.7 mOsm kg⁻¹ = 1 ppt.

Haemolymph osmolality was quantified using nanoosmometry (Charmantier et al., 1998); OC was calculated as the difference between the haemolymph and medium osmolalities. OC was quantified at intermoult; freshly hatched or recently moulted larvae or juveniles were separated from cultures and kept in vessels for 50% of the expected stage duration when they were checked visually (based on preliminary experiments). At the appropriate time, larvae of each stage were placed in petri dishes at the appropriate test salinities and kept for 24 h (see Fig. 1 hereafter). Before proceeding with the measurements, larvae were quickly rinsed in deionized water and gently dried on a filter paper. They were then submersed in mineral oil in order to avoid evaporation and desiccation (any remaining water was aspirated using a first micropipette). Samples of haemolymph (sample volume ~ 30 nl) were taken from the heart by inserting a second micropipette into the body. Adults were placed in individual aquaria and maintained in the test salinities. Prior to haemolymph sampling, their moulting stage was checked through microscopic examination of pleopod setae (Drach, 1939; Drach and Tchernigovtzeff, 1967) and only crabs in intermoult stage C were retained. After 6 days of exposure (see Fig. 1 hereafter), the crabs were rinsed with deionized water and dried with filter paper. Haemolymph was sampled with a micropipette inserted at the basis of a posterior pereiopod, and it was quickly transferred into mineral oil to avoid evaporation. For all experimental stages, haemolymph osmolality was determined with reference to the medium osmolality on a Kalber–Clifton nanoliter osmometer (Clifton Technical Physics, Hartford, NY, USA).

**Data analysis**

For the range of osmolalities between 15 and 39 ppt, data of OC by stage was analysed through two-way factorial analysis of variance (ANOVA) (Underwood, 1997) after confirmation that variance did not show evidence of heterogeneity (Cochran test, P = 0.38) and residuals showed normal distribution (qq-plot of within cell residuals). Both the first juvenile (C I) and adults survived at lower salinities than the larval stages, but we did not record measurements for the C I in seawater. Therefore, the comparison of osmoregulatory capacities was carried out through separate ANOVAs con-
Considering (i) megalopa and first juvenile, and (ii) first juvenile vs. adult. In addition, for adult crabs, we also considered responses under pure freshwater (i.e. 0.03 ppt, shown as < 1 ppt in Fig. 3) and 10 ppt, which were analysed along with the responses at the remaining osmolalities using one-way ANOVA. Differences among treatment combinations were determined using the Student–Newmann–Keuls test.

Results

The percentage survival after exposure to the test salinities for 24 h or 6 days for larvae and juvenile or adults, respectively, is given in Table 1. Recall that different time periods between adults and larvae were needed to achieve stability for measurements of osmoregulation. Differences between those time periods do not explain the contrasting differences in survival rates between larvae and adults. Larvae did not survive at 10 ppt or lower salinities while survival was 60–100% at 20 ppt or higher salinities. Advanced zoeal stages and the megalopa showed moderate to low survival rates at 15 ppt. However, adults survived in all tested salinities until 6 days, even if there was only one survivor in freshwater (< 1 ppt).

Hence, comparisons based on instantaneous mortality rates would show even a stronger contrast between larval and adult mortality at different salinities.

The acclimation time after an abrupt change in salinity was determined in zoea V as a representative for the larval stages, and in adults, using for each of these stages a salinity for which survival was high. We determined the acclimation time for zoea V and adults at the selected salinity (a salinity for which survival was high: preliminary experiments). For zoea V, such tests showed that haemolymph osmolality and OC stabilized after less than 10 hours of exposure; for adults, stabilization occurred after ca. 4–5 days depending on salinity (Fig. 1). The period of exposure of larvae (24 h) and adults (6 days) to the different salinities was established after these tests and was used for the subsequent experiments.

The ability to osmoregulate is illustrated by variations of haemolymph osmolality (Fig. 2) and of OC (Fig. 3) according to salinity for each tested stage. These results revealed significant variations with salinity showing a pattern consistent with hyper- and hypo-regulation (Fig. 2), but such capacity varied considerably among stages (significant interaction stage by salinity: Fig. 3 and Table 2). The zoea I-IV and the megalopa were able to hyper-regulate within the range of 434–581 mOsm kg⁻¹ (15 to 25 ppt) and hyper-osmoconformed in the range of 730–1146 mOsm kg⁻¹ (25 to 38.7 ppt). The zoea V, by contrast showed a weak capacity to osmoregulate, evident only at 581 (20 ppt).

First juvenile crabs (C I) differed in their OC with respect to the megalopa and adults but the difference depended on salinity (Stage:Salinity: comparison with megalopa: \( F_{3,38} = 53.5, P < 10^{-12} \); comparison with adult: \( F_{4,60} = 19.8, P < 10^{-9} \)). At 15 and 20 ppt, juveniles were stronger hyper-regulators than the megalopa while they did not differ significantly in osmoregulation at 25 ppt; in addition, at 39 ppt, juveniles showed on average a weak capacity to hypo-regulate. When compared to adults, juveniles were weaker hyper-regulators. In addition, at 39 ppt the OC was not significantly different from zero: two out of 5 crab I juveniles had positive values of OC (20 and 40) while other two had negative values.

Adults had comparatively the strongest OC of all tested stages (Fig. 3), and, at least 5% managed to survive in freshwater (< 1 ppt), while 20–60% survived at 5 and 10 ppt, respectively (Table 1). They hyper-osmoregulated in the range 434–730 mOsm kg⁻¹ where the OC increased three times with respect to the larval stages. Adults also hypo-osmoregulated at 1146 mOsm kg⁻¹ where survival was 75% (Table 1).

Discussion

We found that Hemigrapsus sanguineus was able to osmoregulate over the entire life cycle, a trait that is likely to contribute
Table 1: *Hemigrapsus sanguineus*: Percent survival of the tested stages according to the different salinities, after exposing each larval stage and the first juvenile for 24 h, and the adults for 6–7 days.

| Osmolality (mOsm kg\(^{-1}\)) | I  | 145 | 290 | 434 | 581 | 730 | 942 | 1146 |
|-------------------------------|----|-----|-----|-----|-----|-----|-----|------|
| Salinity (ppt)                | 1  | 5   | 10  | 15  | 20  | 25  | 32.5| 38.7 |
| ZI                            | 0  | 0   | 0   | 100 | 100 | 100 | 100 | 100  |
| ZII                           | 0  | 0   | 0   | 100 | 100 | 100 | 100 | 90   |
| ZIII                          | 0  | 0   | 0   | 33  | 100 | 100 | 90  | 100  |
| ZIV                           | 0  | 0   | 0   | 33  | 100 | 100 | 100 | 100  |
| ZV                            | 0  | 0   | 0   | 25  | 100 | 100 | 100 | 100  |
| M                             | 0  | 0   | 0   | 30  | 80  | 70  | 60  | 60   |
| Cl                            | NT | NT  | 100 | 100 | 80  | 60  | 40  |      |
| Adults                        | 5  | 20  | 60  | 100 | 100 | 90  | 90  | 75   |

Note: The number of individuals at the start of the exposure was 10 for all larval stages and 5 for juveniles (in the tested salinities); except at 15 ppt for zoea III and IV \((n = 15)\), zoea V and megalopa \((n = 20)\). The number of adults at the start of the exposure was 10 for all salinities, except at freshwater \((n = 20)\), 5 ppt \((n = 15)\) and 15 and 38.7 ppt \((n = 12)\). Survival of adults was 100% at all salinities except at < 1 and 5 ppt: 5 and 20%, respectively (i.e., animals that survived the first 24 h, also survived until the osmoregulation measurements were performed). NT: not tested (due to low number of individuals reaching CI), we prioritized the other salinities; indicated with an asterisk in Fig. 3.

Figure 2: *Hemigrapsus sanguineus*. Variations in the haemolymph osmolality in different lifecycle stages in relation to the osmolality (bottom x axis) and salinity (upper x axis) of the medium at 24°C. Acclimation time was 24 h for larval and crab I stages, and 6 days for adults. Values are shown as average values ± standard error. For zoal stages I–III: \(n = 9–10\), IV–V: \(n = 5\); for megalopa: \(n = 6–8\), for crab I: \(n = 4–5\); for adults: \(n = 9–12\), except for crabs exposed to < 1 ppt: \(n = 1, 5\) ppt: \(n = 3\) and 10 ppt: \(n = 6\). Zoal stages are shown in red (Z I: stars, Z II: diamonds, Z III: triangles, Z IV: squares and Z V: circles); megalopa (M) is shown in green squares; first juvenile crab (CI) is shown in light blue circles and adult is shown in dark blue circles. Note that standard errors may be smaller than symbols.

Figure 3: *Hemigrapsus sanguineus*. Variations in OC at different life cycle stages in relation to the osmolality (bottom x axis) and salinity (upper x axis) of the medium at 24°C. Values are shown as average values ± standard error (replicate numbers as in Fig. 2). Zoal stages (Z I–ZV): red bars; megalopa (M): green bars; Juvenile I crabs (CI): light blue bars; adults crabs (A): dark blue bars; missing bars at < 15 ppt (434 mOsm kg\(^{-1}\)) denote 100% mortality in all larval stages; asterisks instead of bars show salinities not tested for Cl.
is only comparable to the one exhibited by larvae of *Eriocheir sinensis* (Cieluch et al., 2007), another invasive crab found in the North European Seas (Ojaveer et al., 2007). The crab *E. sinensis* is also included in the top 100 invasive alien species of the world (Global Invasive Species Database, 2021). Hence, given the realized invasion of *E. sinensis*, which includes the Baltic Sea, the osmoregulatory pattern reported in this paper is indicative of the strong potential of *H. sanguineus* to establish coastal populations in the newly invaded areas. Whether such full potential is realized over the full life cycle will depend on other factors, such as temperature, which is known to modulate OC in crustaceans (Torres et al., 2021b) and drive salinity tolerance in the larvae of *H. sanguineus* (Epifanio et al., 1998). Hence, whether the full potential is currently realized will depend on latitude, with those local populations present at lower latitude being nearer such potential. However, the realized potential is likely to increase in the future, especially in the Channel and Southern North Seas where isotherms are shifting at a speed of 100 km per decade (Burrows et al., 2011), temperatures have increased at a rate of ∼0.4–0.8°C per decade (Huthnance et al., 2016) and are expected to increase another 2–3°C by 2100 (Schrum et al., 2016). Our data on acute exposure (Table 1) and those of Epifanio et al. (1998) on chronic exposure suggest that larvae of *H. sanguineus* may close the life cycle in habitats of moderately low salinity (> 15 ppt) in situations where larvae experience temperatures in the range of 18–25°C. Adults show a much wider thermal tolerance as shown for instance by populations in the Wadden Sea where winter temperature drops to <10°C.

**Table 2**: *Hemigrapsus sanguineus*: Analysis of variance evaluating the effect of salinity and stage in the haemolymph osmolality during the life cycle

|                  | df | MS      | F    | P    |
|------------------|----|---------|------|------|
| Stage (S)        | 6  | 38 955  | 163  | <10−4|
| Osmolality (O)   | 4  | 125 477 | 526  | <10−4|
| S.O              | 24 | 24 765  | 104  | <10−4|
| Residual         | 280| 239     |      |      |

This analysis considered all zoeal stages, the megalopa and the adult for salinities ranging between 15 ppt (= 434 mOsm kg⁻¹) and 39 ppt (= 1146 mOsm kg⁻¹). Abbreviations: df: degrees of freedom; MS: mean squares, F: Fisher statistics, P: P value.

and in fresh water was lower than that of strong osmoregulators such as *Armases miersii* and *Neohelice granulata* (up to 600 mOsm kg⁻¹ at 1 ppt: Charmantier et al., 1998; Charmantier et al., 2002), which occur respectively in rocky intertidal and estuaries pools.

We also found in the adult a significant capacity to hypo-regulate at high salinities (38.5 ppt). We do not have strong evidence showing that the capacity to hypo-osmoregulate is already present at the first juvenile stage; slight hypo-regulation was detected in two individuals but also slight hyper-regulation was found in other two individuals. In any case, the capacity to hypo-osmoregulate must be clearly established at more advanced juvenile stages. Hypo-osmoregulation in adults was not found by Watanabe (1982) and not studied by Hudson et al. (2018); the latter study did not quantify osmoregulation at concentrated seawater. Differences between our finding and that of Watanabe (1982) may be due to either different temperatures used to acclimatize crabs in the laboratory (this study = 24°C, Watanabe = 15°C), different range of salinities or genetic variation among populations. The pattern of hyper–hypo regulation is however consistent with that found in other species of the same superfamiily (e.g. *Sesarma meinerti*: Gross et al., 1966; *S. curacaoense*: Anger and Charmantier, 2000; *A. miersii*: Charmantier et al., 1998; *N. granulata*: Charmantier et al., 2002).

Most larval stages of *H. sanguineus* (except the zoea V) had the capacity to osmoregulate over the range 15–25 ppt; this pattern and the high survival in response to acute exposure to low salinity (Table 1) are partially consistent with experiments reporting larval salinity tolerance (Epifanio et al., 1998). Such experiments also report a reduction in tolerance of the megalopa, but perhaps the reduction reflects carry-over effects from the zoea V, which showed a reduced capacity to osmoregulate. This ontogenetic pattern of osmoregulation of *H. sanguineus* appears to be intermediate between species showing retention strategy, where the OC is maintained or increased along the larval phase (e.g. Charmantier et al., 1998; Anger and Charmantier, 2000), and those showing export strategy, with osmo-conforming zoea II-IV stages (Charmantier et al., 2002; Cieluch et al., 2004; Anger et al., 2008). Laboratory experiments have shown that *H. sanguineus* larvae exhibit behaviours consistent with an export strategy (Cohen et al., 2015). However, the capacity to osmoregulate in the zoeal stages should provide *H. sanguineus* larvae opportunities to exploit estuarine habitats as no other known crab with such strategy. In those species, the timing of successfully crossing of salinity gradients should be constrained by the re-acquisition of the capacity to osmoregulate (e.g. see Torres et al., 2006) but our study shows that such constraint is weaker in *H. sanguineus*.

The pattern of osmoregulation in *H. sanguineus* compares well with the competitor, the European shore crab *Carcinus maenas*, also with an export strategy (Queiroga and Blanton, 2004). Larvae of both species share a trait that is critical for
invasion in a context of global warming: that tolerance to low salinity increases with temperature (C. maenas: Spitzner et al., 2019; Torres, Thomas, Whiteley, Wilcockson, & Giménez, 2020, 2021a; H. sanguineus: Epifanio et al., 1998). However, H. sanguineus appear to have an advantage in three main points: First, the pattern of hyper–hypo regulation found in the adults for H. sanguineus is not present in C. maenas; hence, hypo-regulation may provide a competitive advantage to H. sanguineus, in intertidal habitats where salinity may increase over summer months at low tide, since it increases survival at high salinity through ion excretion. Second, most larval stages of H. sanguineus osmoregulate while most stages of C. maenas do not (zoea II-IV stages do not osmoregulate: Cieluch et al., 2004). Third, at high temperatures (e.g. 24°C), the capacity to osmoregulate of the zoea I is lower in C. maenas (e.g. zoea I: 55–60 mOsm kg⁻¹; Torres et al. 2021a) as compared to H. sanguineus (~ 100 mOsm kg⁻¹, this study). Thus, in temperate estuaries, at the time of the initiation of the migration from estuarine to open waters, first stage larvae of H. sanguineus are better equipped than those of C. maenas. The advantage of C. maenas over H. sanguineus might occur at low temperatures (≤18°C).

In synthesis, we have found two critical traits that make H. sanguineus stand-up as compared with competitors (including the global invader C. maenas) and with other species with similar life-history strategy and hypo-regulate at high salinity; this combination makes them a species well adapted to intertidal zones and especially for the use of tidal pools at latitudes where salinity can increase during the summer season. Second, the capacity to osmoregulate is present along most of the larval phase, which provides opportunities for use and invasion of estuarine waters.

**Authors’ contributions**

GT, LG and GC conceived the experimental design. GT performed the larval rearing. GC performed the OC determinations. GT, LG and GC analysed the osmoregulation data. GT and LG wrote the first draft. All authors contributed to the writing of the manuscript and gave final approval for publication.

**Compliance with Ethical Standards**

The research presented in this paper complies with the guidelines from the directives 2010/63/EU of the European parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes.

**Data accessibility**

All data for this paper will be available from PANGAEA Data Publisher https://www.pangaea.de.

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