

## Article

# Euryhalinity and Geographical Origin Aid Global Alien Crayfish Invasions

Aldona Dobrzycka-Krahel <sup>1,\*</sup>  and Maria Leonor Fidalgo <sup>2</sup><sup>1</sup> Business Faculty, WSB University in Gdańsk, Al. Grunwaldzka 238 A, 80-266 Gdańsk, Poland<sup>2</sup> Department of Biology, University of Porto, Rua do Campo Alegre, s/n, 4169-007 Porto, Portugal

\* Correspondence: adobrzycka@wsb.gda.pl

**Abstract:** Salinity tolerance is a determinant of a narrow or wide distribution range of organisms. Crayfishes are important key species in many aquatic environments so require a better understanding of their ability to live in different saline regimes. We identified all alien crayfish and examined their habitats (freshwater and/or saline) and origins to test whether these factors predict their dispersal. We used contingency tables populated with raw frequency data with  $\chi^2$ -tests and assessed statistical significance at  $\alpha$  of 0.05. We identified 21 alien crayfishes and we found that alien crayfish species were disproportionately freshwater (71%), with significantly lower proportions of euryhaline crayfishes inhabiting freshwater to saline environments (29%). Alien crayfishes also significantly disproportionately originate from America (67% of these taxa) when compared to all 'other' grouped regions (33%). In total, 36% of American crayfishes represent euryhaline species inhabiting freshwater to saline habitats against only 14% of crayfishes from all "other" grouped regions. This suggests that binomial euryhalinity/origin can help understand the potential of spread. We discussed obtained results with known experimental data on salinity tolerance, osmoregulation, growth, and reproduction of American alien crayfish. The paper will help in the management of crayfish spread.

**Keywords:** alien crayfish; global change biology; salinity tolerance



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## 1. Introduction

Aquatic species are generally divided into living in fresh and in saline waters (brackish and/or marine environments). Freshwater species usually do not invade saline habitats. Salinity tolerance specifies their ability to adapt to a wide or narrow range of occurrences. Our basic knowledge is based on the fact that the "normal" environment of crayfish is usually connected only with fresh waters and crayfish are considered stenohaline freshwater organisms, inhabiting a narrow range of low-salinity environments.

In fact, when we refer to crayfish, we are talking about decapods that are distributed on all continents except for the Antarctic continent, the Indian subcontinent, and the African mainland [1,2].

Freshwater crayfish constitute a monophyletic group of described species, which are arranged into four families: Astacidae, Cambaridae, Cambaroididae, and Parastacidae [3]. They have two centres of radiation: one in the Southeastern Appalachian Mountains in North America and the others in Southeastern Australia [2]. They inhabit a variety of freshwater environments from streams and rivers to ponds and lakes, but in some countries, they have been found inhabiting saline coastal waters [4]. They even occur in coastal landlines and oceanic islands such as Cuba, Madagascar, and New Guinea, and are represented by over 640 species with an average of 5–10 species still being described each year [2]. The ability of crayfish to tolerate elevated salinities could arise from their descent from a common marine ancestor (185–225 million years ago) [2,5]. Their tolerance to constantly low or variable salinity levels is partially achieved through cells specialized

in the process of ionic regulation that compensate for the loss of ions caused by the osmotic gradient between the haemolymph and the surrounding medium by active uptake of  $\text{Na}^+$  and  $\text{Cl}^-$  [6]. In addition, the biological cycle of crustaceans includes various development stages, so it is expected that their establishment in new habitats may depend on the ability of each of their development stages to adapt to salinity and its rapid changes [7,8].

Nowadays in Europe, some crayfish species from America (e.g., *Faxonius limosus*, *Pacifastacus leniusculus*, and *Procambarus clarkii*) become the most widely spread non-indigenous crayfish species) [9,10].

Due to knowledge gaps about the salinity tolerances of crayfishes originating from different geographical regions, the aim of this investigation was to compare the euryhalinity of American crayfishes to other nonindigenous crayfishes. We hypothesized that American crayfishes are generally more euryhaline than those from other geographical regions, which has facilitated the former's growing distribution. Euryhalinity refers to broad salinity tolerance (capability of surviving in both freshwater and saline water). Species (including crayfish) may vary in their range of tolerable salinity levels, which may determine their distribution.

The aims of this paper were:

1. to analyse whether the euryhalinity of American crayfishes was higher than that of crayfishes from other geographical regions;
2. to determine global alien crayfish invasion in both fresh and saline waters, and assess their ability to tolerate, osmoregulate, reproduce and grow in different saline regimes.

## 2. Materials and Methods

Based on our reanalysis of known scientific literature, we examined the distribution of crayfish in both freshwater and saline environments. Later (on 1 October 2021), we downloaded the alien invertebrate species from the database GRIIS (Global Register of Introduced and Invasive Species) [11]. We also searched for the crayfish species and their geographical origins in CABI (Centre for Agriculture and Bioscience International) and GBIF (Global Biodiversity Information Facility) databases [12,13].

Information about the habitats of those species (freshwater or saline) was obtained from CABI, GRIIS, and NEMESIS (Smithsonian Environmental Research Center's National Estuarine and Marine Exotic Species Information System) [14]. Salinity categorizations (freshwater, less than 0.5‰) and saline (brackish, 0.5–30‰), and marine (greater than 30‰) [15] were obtained for each species. We statistically tested the null hypothesis that the proportions of American crayfish species and crayfish from other geographical origins (1) each are distributed approximately equally among their relative salinity adaptive zones: either (a) freshwater, (b) both freshwater and saline (indicating euryhalinity), and (2) these proportions are approximately equal between the two geographical origin groups (American versus 'other'). We used contingency tables populated with raw frequency data with  $\chi^2$  tests and assessed statistical significance at  $\alpha$  of 0.05 using the STATISTICA 13.1 PL program (StatSoft).

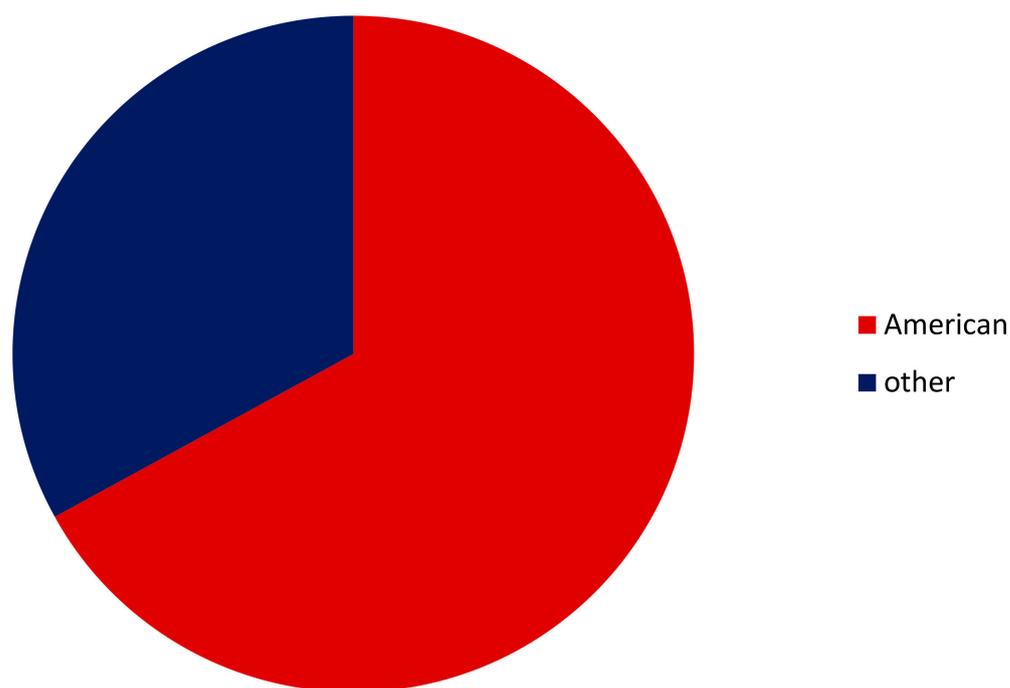
## 3. Results and Discussion

### 3.1. Alien Crayfishes

The contribution of American versus crayfish coming from other geographical areas is shown in Figure 1.

American crayfishes are the most common alien crayfish species in the world (Figure 1), representing 67% against 33% from the other areas of origin, namely Europe and Australia.

The geographical distribution of alien crayfish species in freshwater and/or saline habitats is given in Table 1.



**Figure 1.** Proportion of American versus crayfish coming from other geographical origins, based on GRIIS [11].

**Table 1.** Alien crayfish species based on GRIIS (Global Register of Introduced and Invasive Species) [11]. Distribution in freshwater and/or saline habitats determined due to CABI (Centre for Agriculture and Bioscience International), GBIF (Global Biodiversity Information Facility), GRIIS and NEMESIS (Smithsonian Environmental Research Center’s National Estuarine and Marine Exotic Species Information System) databases [11–14].

	Species	Origin [12,13]	Habitat	
			Freshwater [11]	Saline [11,12,14]
1	<i>Austropotamobius fulcisanus orientalis</i> (Karaman, 1929)	Europe	+ (I)	–
2	<i>Astacus astacus</i> (Linnaeus, 1758)	Europe	+ (UK)	–
3	<i>Cherax albidus</i> (Clark, 1936)	Australia	+ (SA)	–
4	<i>Cherax destructor</i> (Clark, 1936)	Australia	+ (Ch, G, S, M, SA)	–
5	<i>Cherax quadricarinatus</i> (von Martins, 1868)	Australia	+ (A, B, Ch, C, G, Eq, Is, Ja, Ma, Me, Mal, Na, P, Ph, Pa, Si, Slo, Th, Tri, V, SA, Z, Zi)	–
6	<i>Cherax tenuimanus</i> (Smith, 1912)	Australia	+ (Ch, G, Mau, Mal, NZ, Pan, P, T, SA)	–
7	<i>Faxonius immunis</i> (Hagen, 1870)	North America	+ (G, F, Sw)	–
8	<i>Faxonius juvenilis</i> (Hagen, 1870)	North America	+ (F)	–

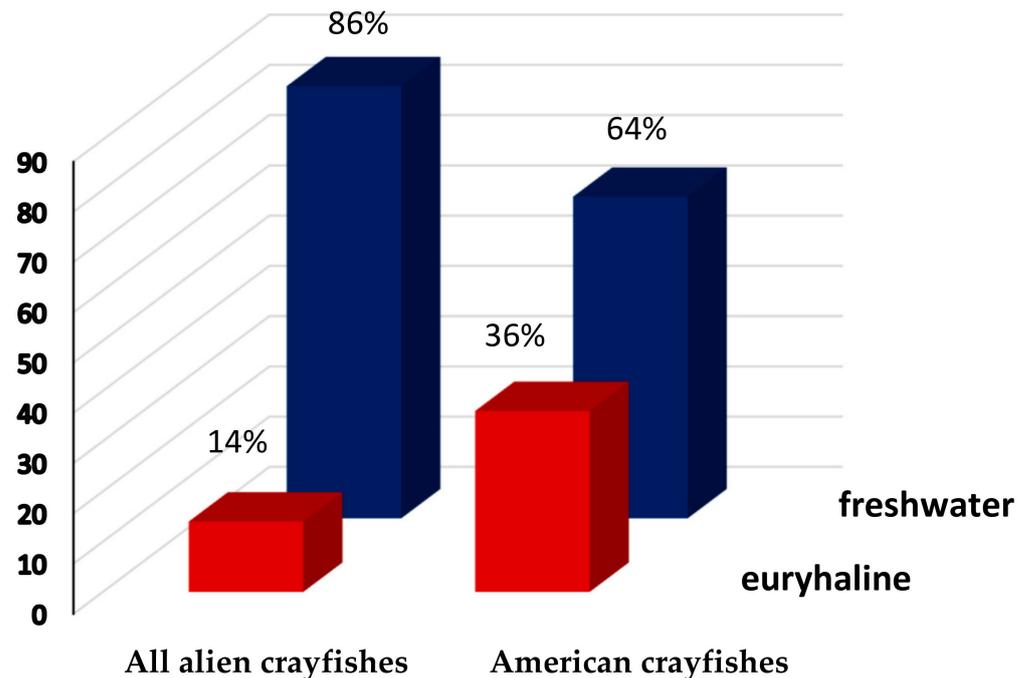
Table 1. Cont.

	Species	Origin [12,13]	Habitat	
			Freshwater [11]	Saline [11,12,14]
9	<i>Faxonius limosus</i> (Rafinesque, 1817)	North America	+ (Au, Be, Bel, Cro, F, G, Hu, I, La, Lux, Li, E, Mar, Ne, Po, Ro, S, Se, Slo, Sl, Sw, Swit, UK)	+ [12]
10	<i>Faxonius obscurus</i> (Hagen, 1870)	North America	+ (Ca)	–
11	<i>Faxonius rusticus</i> (Girard, 1852)	North America	+ (Sw)	–
12	<i>Faxonius virilis</i> (Hagen, 1870)	North America	+ (Sw)	+ (USA) [14]
13	<i>Pacifastacus leniusculus</i> (Dana, 1852)	North America	+ (Au, Be, Swit, Cz, G, D, E, S, Fi, F, UK, Gr, Cro, Hu, I, J, Li, Lux, La, No, Po, Por, Ro, Sw, Slo, Sl)	+ (USA) [12,14]
14	<i>Pontastacus (Astacus)</i> <i>leptodactylus</i> (Eschscholtz, 1823)	Eastern Europe	+ (Be, Ma, Ne, Sw, Swit, U, Cz, G, D, Fi, UK, I, La, R)	+ (Swit, U, Cz, G, D, Fi, F, UK, I, La, R) [11]
15	<i>Procambarus acutus</i> (Girard, 1852)	North America	+ (UK, Ne, Sw, Eg)	–
16	<i>Procambarus alleni</i> (Faxon, 1884)	North America	+ (Eq)	–
17	<i>Procambarus clarkii</i> (Girard, 1852)	North America	+ (Sw, A, Be, Br, Bl, Swit, Ch, CR, C, G, DR, Eq, Eg, S, F, UK, Ge, Gu, Is, I, J, K, Mar, Ma, Ni, Nic, Ne, Pan, Ph, Po, Por, Su, Th, Ug, Ve, V, SA, Z, Zi)	+ (Au, Be, Br, Bl, Swi, Ch, Co, CR, C, G, Do, Eq, Eg, S, F, UK, Ge, Gu, Is, I, J, K, Ma, Ni, Nic, Ne, Pan, Ph, Po, Por, Su, Th, Ug, Ve, V, SA, Z, Zi) [11]
18	<i>Procambarus cubensis</i> (Erichson, 1846)	Central America	+ (G)	+ (G) [11]
19	<i>Procambarus fallax</i> (Hagen, 1870)	North America	+ (G, E, Ne, Po, Ro, U, Sw)	–
20	<i>Procambarus virginialis</i> (Lyko, 2017)	North America	+ (Mad, Mal, Ne)	–
21	<i>Procambarus zonangulus</i> (Hobbs and Hobbs III, 1990)	North America	+ (Eg)	–

Notes: Abbreviations: + Present/– Absent. (A-Argentina, Au-Austria, B-Bahamas, Be-Belgium, Bel-Belarus, Bl-Belize, Br-Brazil, Ca-Canada, C-Colombia, C-Cyprus, Ch-China, CR-Costa Rica, Cro-Croatia, Cz-Czechia, D-Denmark, Do-Dominica, DR-Dominican Republic, E-Estonia, Eg-Egypt, Eq-Ecuador, F-France, Fi-Finland, Ge-Georgia, G-Germany, Gr-Greece, Gu-Guatemala, Hu-Hungary, I-Italy, Is-Israel, Ja-Jamaica, J-Japan, K-Kenya, La-Latvia, Li-Lithuania, Lux-Luxemburg, Mad-Madagascar, Mal-Malaysia, Ma-Malta, Mau-Mauritius, Mar-Morocco, Na-Namibia, Ne-Netherlands, Nic-Nicaragua, Ni-Nigeria, No-Norway, M-Mexico, NZ-New Zealand, Pan-Panama, Pa-Paraguay, Ph-Philippines, Po-Poland, Por-Portugal, P-Peru, Ro-Romania, R-Russian Federation, SA-South Africa, Se-Serbia, Si-Singapore, Slo-Slovenia, Sl-Slovakia, S-Spain, Su-Sudan, Swit-Switzerland, Sw-Sweden, Th-Thailand, Tri-Trinidad and Tobago, T-Tunisia, Ug-Uganda, UK-United Kingdom, U-Ukraine, USA-United States of America, Ve-Venezuela, V-Vietnam, Zi-Zimbabwe, Z-Zambia).

### 3.2. Euryhalinity of All Alien versus American Crayfishes

In total, 21 crayfish species registered in GRIIS [11] were assigned as aliens in the world. 86% of them tolerate only freshwater habitats. The majority of alien crayfish species (67%) (Figure 1) are of American origin. Among them 64% are freshwater and 36% are euryhaline and can live in fresh to saline habitats (Figure 2).



**Figure 2.** Proportions of global alien crayfish species versus American crayfish species living in freshwater and fresh to saline habitats.

Significantly disproportionately more alien crayfish species live in freshwater habitats, compared to euryhaline crayfish living in freshwater to saline waters ( $\chi^2 = 17.64$ ,  $df = 1$ ,  $p = 0.000027$ ). Similarly, significantly disproportionately more alien crayfish species originate from America compared to ‘other’ regions ( $\chi^2 = 11.56$ ,  $df = 1$ ,  $p = 0.000674$ ). Additionally, significantly disproportionately more alien crayfish of American origin live in fresh to saline waters ( $\chi^2 = 40.19934$ ,  $df = 1$ ,  $p < 0.001$ ) than in freshwater.

### 3.3. Distribution of Invasive Alien Crayfishes That Can Live in Freshwater and Saline Environments Based on Field Observations

Crayfish species that can live in wide salinity regimes from freshwater to saline environments include six species: one *Pontastacus (Astacus) leptodactylus* of Eastern European origin and five American species (*Faxonius limosus*, *Faxonius virilis*, *Pacifastacus leniusculus*, *Procambarus clarkii*, *Procambarus cubensis*).

A brief description of the worldwide distribution of these species is presented below. The narrow-clawed crayfish *Pontastacus (Astacus) leptodactylus* is also referred to as *Astacus leptodactylus* and we will adopt this name from now onwards. *A. leptodactylus* is native to Eastern Europe and inhabits freshwater and saltwater habitats in several countries as shown in Table 1 and according to Souty-Grosset et al. [16] who reported over 30 countries in Europe and some Asiatic countries.

Other euryhaline crayfishes are of American origin:

*F. limosus* (syn. *Orconectes limosus*) can live both in freshwater and in saline environments. In the freshwater environments it has been reported from 22 European countries according to Kouba et al. [9] and also shown in Table 1. In saline habitats, it has been observed from the Curonian Lagoon [17], and the Polish coastal zone of the Baltic Sea: brackish lagoons, coastal lakes, and river mouths) [10,18–24].

*F. virilis* (syn. *Orconectes virilis*) has a broad distribution and occurs from the southern tip of the Hudson Bay, southward from New England to western Montana, and through the Mississippi, Missouri, and Ohio River basins to northern Arkansas and Oklahoma. In Oklahoma State, this species is found in the Cimarron and Arkansas River drainages in the northern two-thirds of the State [13]. It is the most abundant crayfish in Ptarmigan Bay (Canada) [25]. It also occurs in Ontario lakes [26]. This species has a broad native range

and was considered the most widespread crayfish species in Canada and the United States of America [9].

However, recent phylogenetic, and phylogeographic studies revealed that it is actually a diverse species complex [27,28]. The genetic analysis of European populations suggested that they represent a lineage distinct from *F. virilis* in a strict sense [29], so its distribution is narrower than shown in Table 1.

The signal crayfish *P. leniusculus* occupies a wide range of habitats including freshwater streams, rivers, and lakes in the USA, Austria, Canada, Cyprus, Czechia, England, Finland, France, Germany, Greece, Japan, Latvia, Lithuania, Luxemburg, Netherlands, Poland, Slovenia, Spain, Sweden [16,30,31]. Records of the signal crayfish in Norway, Slovakia [32], Croatia [33] and Portugal [34,35] extend the list of Souty-Grosset et al. [16] and the list is given in Table 1. This species was also reported in coastal environments on the west coast of North America, it is a common inhabitant of northern Californian coastal riverine environments and is found regularly in brackish-water areas, occasionally carrying barnacles on the exoskeleton [36] and in coastal waters in the Danish part of the Baltic Sea as well [37].

Regarding *P. clarkii*, its natural range occurs in Mexico and the southern USA. It is widely distributed and naturally occurs along northward along the Mississippi River to southeastern Missouri and southwestern Illinois and the Gulf Coastal Plain from northeastern Mexico to the Florida panhandle [38,39]. In the State of Oklahoma, *P. clarkii* occurs naturally in the extreme southeastern corner where the Gulf Coastal Plain reaches into this US State. This species inhabits a wide variety of habitats, including swamps, flooded ditches, and creeks [13]. Sharfstein and Chafin [40] suggested that *P. clarkii* could be cultured in groundwater with a salinity of 12‰.

It was introduced for the first time on the Iberian Peninsula, in the 1970s for aquaculture purposes [41]. Now, the number of territories invaded by *P. clarkii* is huge (Table 1). This species can be found both in freshwater and saline ecosystems. It is present in a shallow Mediterranean coastal lagoon in the Albufera Natural Park, Spain [42]. Scalici et al. [43] demonstrated that the *P. clarkii* population was capable of living and reproducing in a brackish wetland in Italy, with salinity varying between 16.2 and 29.6‰.

In Portugal, the red swamp crayfish were recorded for the first time in 1979 at the Caia River, in southern Portugal [44]. Nowadays this species is well-established in most Portuguese river basins, including a shallow lagoon in the Aveiro region [45]. Its presence was also reported on the island of São Miguel (Azores, Portugal) [46]. Recently the species was detected in Krasieński Garden and Żerań Canal in Warsaw (central Poland) [47].

The Cuban crayfish *P. cubensis* can live both in fresh and in brackish waters in Germany [11]. This species is native to Cuba [48] being known to occur in lotic and lentic habitats, and mountain streams [49]. It was introduced to Moscow (Russia) in the 1970s [50,51]. This alien crayfish has been found in the pet trade of several European countries (e.g., Czechia, Hungary, and Ukraine) [52–54].

### 3.4. Is Salinity a Barrier to the Dispersal of Crayfishes?

Salinity is one of the most significant factors limiting the distribution of species in aquatic environments [55]. So, when marine organisms enter freshwater habitats must evolve to retain osmotic levels in body fluids, which requires high energetic costs, while freshwater taxa entering marine environments must evolve to maintain lower body fluid concentrations relative to the highly concentrated environment [56,57]. However, despite physiological stress and the energetic costs imposed on alien species when they enter a new habitat some alien marine and brackish water species have established in estuaries and managed to overcome the salinity barrier between fresh and brackish waters.

In fact, salinity and its variations are among the main abiotic factors that influence essential processes of aquatic animals (namely crayfish) such as feeding, growth, and reproduction, which determine their survival and success in aquatic environments [6,7,58–61].

For this reason, crustaceans developed several strategies (e.g., behavioural, ecological, molecular, and physiological mechanisms) to cope with salinity changes [62].

On the other hand, salinity tolerance may be of high relevance for the spread of alien species into new locations due to, for example, possible dispersal to new watersheds through different estuaries, thus functioning as biological corridors [63]. As mentioned by several researchers, e.g., [23,64,65] some freshwater organisms can migrate through saline ecosystems, but due to physiological constraints, certain crustaceans are sensitive to high salinity values, namely during specific parts of their biological cycle such as during the juvenile stage and reproductive periods [7,8,66]. For example, a laboratory study carried out on the tolerance of the green crab *Carcinus maenas* larvae facing hypo-osmotic stress showed that a salinity of at least 25‰ is needed for its successful development [7]. In contrast, at lower salinities (20‰), significant decreases were found in the rates of early zoeal survival, growth, development, assimilation and respiration [7], being thus in line with the findings of Cieluch et al. [6] that confirmed a change of osmoregulation pattern in *C. maenas* throughout its biological cycle.

In turn, Schram [67] pointed out that crayfishes have a long history in temperate fresh waters stretching back millions of years and emerged from marine ancestors about 200 million years ago. Therefore, in structural and physiological terms, crayfish have had enough time to become adapted to hypoosmotic environments, although some species have maintained the ability to survive in brackish waters [68,69]. Despite their preference for freshwater habitats, some crayfish species are euryhaline, and certain living species have retained the ability to tolerate higher salinities [70] such as the red swamp crayfish *P. clarkii* [71–73]. The signal crayfish *P. leniusculus* is a common species in coastal waters of northern California, being thus considered a euryhaline species, which also inhabits saline waters [66,74,75]. Moreover, this species is regularly observed in brackish areas, with barnacles being transported on the carapace [36], denoting a rather prolonged stay in such areas.

### 3.5. Experimental Evaluation of Salinity Tolerance and Osmoregulation

When addressing the issue of salinity in the larval biology of decapod crustaceans some negative effects associated with osmotic stress were mentioned by Anger [8], namely delayed development, a decrease in survival, feeding and growth rates, which can be coupled with extended or suspended moulting cycles as well as with metabolic and behavioural changes.

As far as crayfish are concerned, these decapods have adapted well to freshwater and they can spend their whole life in freshwater habitats, including reproduction and development, although the degree of adaptation to freshwater appears to vary between different species. For example, the allochthonous *P. leniusculus* can better survive in seawater than the European crayfish *A. leptodactylus* although 75% and 100% mortality was recorded after two and three weeks at 28 and 35‰ salinity, respectively [66].

Under natural conditions, most crayfishes have adopted a stenohaline way of life, although they can cope with some degree of experimentally increased salinity [66,69,74,76], and a large number of species are known to live in brackish habitats e.g., [60,66,77]. Laboratory tests carried out with *P. leniusculus* confirmed that adult animals were able to survive in 28‰ over nine weeks but were not reported to reproduce in high salinity waters [66].

Owing to economic purposes (e.g., aquaculture, pet market, fishing bait) and due to environmental concerns, several crayfish species have been the target of studies related to the evaluation of saline tolerance and osmoregulatory capacity. For example, it was reported that *A. leptodactylus* lives in waters up to salinities of 14‰ in the Caspian Sea and feeds on brackish organisms, which means that *Astacus* spp. stay long enough to feed on these prey [77]. Experimental results provided by Holdich et al. [66] showed that adults and juveniles of *A. leptodactylus* are well adapted to survive in salinities of at least 21‰ in long-term exposure and to be transferred directly back into fresh water to breed. Köksal [78] reported this species from the Baltic coast and the Black and Caspian Seas.

Experiments showed that adults were able to survive salinities of 28‰ over nine weeks, although their ability to survive in full salinity was less than that of *P. leniusculus* [66].

As underlined by Holdich et al. [66] adults of *A. leptodactylus* and *P. leniusculus* species showed a similar osmotic response when transferred to a range of seawater concentrations (salinities of 7, 14, 21 and 28 where 100% seawater equals 35) for 48-h periods. The osmolality of the haemolymph increased significantly as the salinity increased. These species hyper-regulated in freshwater and lower salinities (7‰ and 14‰) than the haemolymph concentration, and hyporegulated in higher salinities (21‰ and 28‰) than the haemolymph concentration, although some slight differences in terms of the transition from hyper- to hyporegulation were detected between the two species.

Concerning the osmoregulation capacity of *A. leptodactylus* Susanto and Charmantier [76] mentioned that this species was increasingly able to hyperregulate in the range of water salinities 13–17‰, being thus in line with data of Yildiz et al. [79] who pointed out that the species was able of hyper-regulate in 10‰ for 96 h. Similar results were also obtained for *P. leniusculus* when exposed to 7‰ and 14‰ for three and six weeks, showing a high efficiency in terms of osmoregulation of its body fluids [66].

Based on the assessment of the effects of salinity on moulting of *A. leptodactylus* exposed to a wide range of salinities (2, 4, 6, 8, 10, 12, and 14‰) no significant differences were observed between 10, 12 and 14‰, although the best salinity corresponded to 10‰, in which most specimens moulted quickly and easily [80]. However, the response of *A. leptodactylus* when specimens were directly transferred from freshwater to 10‰, resulted in physiological stress expressed by an increase in haemolymph glucose levels together with changes in the concentration of some ions [79]. Increased release of glucose into circulation may enable the functions of basic metabolic processes during energy-requiring conditions, allowing organisms to cope with stress. As mentioned by Jussila et al. [81] elevation in haemolymph glucose may represent a secondary stress response and a useful tool in assessing stress in crustaceans. This might be the case of *A. leptodactylus* which has the capacity to tolerate a wide range of salinity conditions, but considering recorded changes of other haemolymph parameters (e.g., total protein, calcium, magnesium, sodium, and chloride) its exposure to saline water for 96 h may represent a source of stress, recommending, therefore, the realization of long-term studies to establish the respective effects on its life cycle [79].

Taking into account the high invasive potential of *P. clarkii* due to its high ecological plasticity, this species has motivated a lot of research related to saline tolerance and/or osmoregulatory capacity, e.g., [71–73]. Laboratory tests to investigate whether this species could breed, moult and live in water with different salinity levels were carried out by Casellato and Masiero [71] by exposing adult individuals for 100 days to a range of water salinity varying from 5 to 33‰. These authors reported that animals survived during the whole experiment period (100 days) at salinity concentrations up to 25‰, regularly moulted and mated, showing that *P. clarkii* may invade estuarine and brackish habitats on the Adriatic coast.

In another study, aiming to simulate real conditions in nature where crayfish gradually conquer brackish waters instead of sudden colonization, *P. clarkii* specimens were exposed for 54 days to a gradual application of salt starting from 0.1–0.2‰ in control water up to 35‰ [72]. In this laboratory experiment, a survival rate of 96% was registered in such high salinity and surviving animals appeared to be in good health during the exposure period.

More recently, Dörr et al. [73] also attempted to simulate the natural transition from freshwater (0.1–0.2‰) into seawater by exposing adults of *P. clarkii* for 65 days to increasing salt concentrations up to 35.3‰ that is a similar salinity to that of Mediterranean Sea. On this laboratory test mortality was relatively low and 80% of males and more than 60% of females, were maintained at increasing values of salinity up to 35.3‰. It is known that *P. clarkii* can cope with salinity stress [43,60,71,72], but there is still little information about changes in terms of antioxidant mechanisms counteracting the production of reactive oxygen species (ROS) when exposed to a wide-range salinity. Aiming to overcome

this gap of knowledge oxidative stress biomarkers were assessed by Dörr et al. [73] on hepatopancreas and gills of the red swamp crayfish (e.g., catalase, glutathione peroxidase, malondialdehyde, superoxide dismutase) and the obtained results suggested a great potential of *P. clarkii* to cope with salinity stress. The two organs showed high levels of antioxidant biomarkers, but mainly the hepatopancreas [73] reflecting, for example, the changes linked to the biological cycle or the effect of chemical physical stressors [82]. As mentioned by Dörr et al. [73], *P. clarkii* has shown great resistance and adaptability to laboratory conditions and the obtained results denoted its possibility to colonize new habitats by moving from rivers towards brackish waters and even by migrating upstream from the estuarine zone.

In turn, Vesely et al. [61] addressed the salinity tolerance of marbled crayfish *P. fallax f. virginialis* by conducting a 155-day experiment. Specimens were acclimated in a step-wise manner for five days to a range of salinities (6, 9, 12, 15, and 18‰). The results of this study confirmed that *P. fallax* has a lower salinity tolerance compared with other crayfish species, which may limit their invasive potential in brackish environments.

Regarding the osmoregulatory capacity of crayfish, note that the antennal gland (i.e., the main excretory organ able to maintain osmotic homeostasis) plays an important role in terms of osmoregulation and hydric balance [83]. Histomorphological observations on the antennal gland of *P. clarkii* may explain the observed modification of haemolymph concentrations as reported by Bissattini et al. [72]. Moreover, as mentioned by Sarver et al. [84] structural alterations make the antennal glands of this freshwater crayfish similar to those of marine decapods whose urine is isoosmotic with the haemolymph.

### 3.6. Experimental Evaluation of Growth in Fresh and in Saline Waters

Crayfish growth is a complex process involving a series of moult and intermoult periods that are under the control of several hormones. Moulting is a stressful process for crustaceans due to the high metabolic costs involved in this process [85]. Many crayfish species can survive in saline waters for a short period, while long time exposure to high salinity values will have adverse effects on growth and survival. However, exposure to small amounts of salt may be beneficial to the growth of crayfish *P. clarkii* because energetic costs to maintain salt and water balance are reduced [72].

In turn, juveniles and adults of *A. leptodactylus* are well adapted to survival in salinities of at least 21‰ in the long term, and to be transferred directly back into fresh water [66]. However, other researchers stated that crayfish's ability to colonize the estuarine environment may be restricted to areas of low salinity [76] due to the adverse effects of seawater on egg development and hatching. In the same sense, Meineri et al. [60] reported that juveniles of *P. clarkii* growth and reproduction were proved to be affected negatively when salinity was above 5 g L<sup>-1</sup> in Camargue (delta of the River Rhône, Southern France). Moreover, temporary water bodies within the salinity range for *P. clarkii* are used as safe sites for its reproduction and for this reason a significant reduction in freshwater inputs to the Camargue region might be important to restore a higher salinity level and consequently reduce the abundance, expansion and reproductive success of *P. clarkii* in this important wetland [60].

Other laboratory studies carried out on different crayfish species also confirmed a similar trend, although with some variability. For example, the spiny cheek crayfish *F. limosus* failed to successfully reproduce and grow when salinity was above 7‰ [23]. In the case of *P. leniusculus* and *A. leptodactylus*, their eggs could not survive at salinity higher than 14‰ [66]. Still, regarding *A. leptodactylus*, data provided by Hesni et al. [80] reported that most specimens moulted easily and quickly when exposed to a salinity of 10‰, being thus approximately in line with Holdich et al. [66].

### 3.7. Experimental Evaluation of Reproduction in Fresh and in Saline Waters

Adverse effects of salinity on the reproduction, growth, development, and survival of crayfish are known, but some species have shown the capacity to live in estuarine

habitats due to their osmoregulation capacity and antioxidative mechanisms, the main responses to salinity changes. For instance, *P. clarkii* can breed in brackish water where its presence is increasingly more frequent [43,71]. However, data from Meineri et al. [60] in the Camargue wetland suggested that low salinity values increase the occurrence, abundance and reproduction performance of *P. clarkii* and juvenile abundance was affected negatively when salinity was above 5 g L<sup>-1</sup>.

Yet the signal crayfish *P. leniusculus* tolerates saline water to some extent, e.g., [61,66], but has not been reported to reproduce in high salinity waters due to the negative effects of seawater on egg development and hatching [66,76].

#### 4. Conclusions

From a geographical point of view, in America, crayfish can live in highly changeable saline conditions [14]. Indeed, the diverse historical environmental regimes, which include fresh and saline environments experienced by taxa native to this region may provide an evolutionary predisposition to invade freshwater to saline habitats. Moreover, changing environmental conditions in recipient areas, caused by the salinisation of freshwaters due to anthropogenic and geologic sources [86] constitute suitable habitats for salt-tolerant species such as American crayfishes. Euryhalinity seems to be beneficial for crayfishes not only as the trait that facilitate their dispersal but from the behavioural point of view because may help in, for example, escaping predators. Moreover, the utilization of different salinity conditions by euryhaline species may represent an important advantage over stenohaline ones if the benefits of moving to outweigh the metabolic costs of osmoregulation, namely in case of providing additional feeding resources, reducing competition, facilitating the use of certain areas for reproduction. Thus, our results showed that significantly more alien crayfish species originate from America compared to 'other' regions and more alien crayfish of American origin live in fresh to saline waters than in freshwaters.

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