

Research Article

Morphological features of the Oriental river prawn (*Macrobrachium nipponense* (de Haan, 1849)) at different localities in Ukraine

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Abstract

The Oriental river prawn (*Macrobrachium nipponense*) is an East Asian decapod, recently recorded as invasive in Europe. Here, we describe key morphological and morphometric features (sex, total length and weight, lengths of both 2nd pereopods and number of dorsal and ventral rostrum spines) for distinguishing populations in different waterbody types in Ukraine (one brackish and two freshwater). Over the year, males tended to slightly dominate with a mean catch of 54.79%. Females became more prevalent in catches during the June–October spawning period when ovigerous individuals were recorded, a pattern that differed from both tropical and temperate parts of its native range. We recorded no significant difference in linear growth characteristics between the invasive prawns and native populations, and both sexes displayed a stable mass-length correlation with isometric growth characteristics typical of the species. Our results indicated that dentition number (dorsal and ventral) was the most stable morphological feature, while the other parameters examined could be used for both population tagging and observation of seasonal changes. In Ukraine, female prawn size was greater in freshwaters than brackish waters, opposite to the trends observed in its native range. The larger size and growth rate of prawns in the Danube delta suggests that brackish water bodies in Ukraine have a relatively low potential for fisheries and prawn farming.

Key words: invasive range, sex ratio, morphology, Decapoda, Danube delta, estuaries

Introduction

The commercial exploitation of crustaceans hold considerable interest for industrial applications in the food and pharmaceutical sectors, given their high protein and nutritional values (Bondad-Reantaso et al. 2012). Over recent years, aquaculture has proven an effective method for ensuring a stable supply in the food production sector and reducing pressure on natural populations (Wickins and Lee 2008; Anh et al. 2011). Both prawn fisheries and farming are now important high-tech industries with high demand in the global market and, as such, provide a significant economic contribution (Tlustý 2002; Primavera and Esteban 2008). The total volume of aquaculture production in 2020 was 122.6 million tonnes of live weight. This number includes 87.5 million tonnes of aquatic animals, mainly intended for human

consumption, 35.1 million tonnes of algae for food and non-food use, and 700 tonnes of shells and pearls for decorative purposes (FAO 2020).

Studying the population characteristics of commercial species improves our understanding of their population structure dynamics, abundance and density. It also contributes to the assessment of impacts on aquatic ecosystems, providing the basis for sustainable use of resources and maintenance of ecosystem balance (Royama 1981; Schaefer 1991; Gardner et al. 2020). This is of especial importance in the case of alien invasive species, which may have higher trait plasticity than local species (Grotkopp et al. 2002; Mason et al. 2008; Leishman et al. 2010), potentially increasing their impact on local ecosystems (Morris and Akins 2009; Çınar et al. 2014; Albins 2015). Commercial exploitation represents one potential method for controlling such alien species by reducing their number and minimising potential negative impacts (Lafferty and Kuris, 1994; Thresher and Kuris 2004).

The Oriental river prawn, *Macrobrachium nipponense* (de Haan, 1849), is a decapod species distributed throughout Southeast Asia, being found in China, Japan, Korea, Vietnam, Taiwan and Myanmar (Kusamura and Suzuki 1997; Cai and Dai 1999; Chen et al. 2017; Zheng et al. 2019). In their native range, the species is widely targeted in the fisheries industry, including aquaculture (Kong et al. 2014; Lavajoo et al. 2019; Hsiung et al. 2021). Many countries, including China, Vietnam and Indonesia, already carry out commercial cultivation and successfully export the species on the world market (Zhang et al. 2020). Overall, the species shows rapid growth, is tolerant of poor environmental conditions and the product is considered quality and safe (Kutty and Weimin 2010).

In recent years, there have been numerous cases of Oriental river prawn introductions around the world. In the Middle East region, the species is known to inhabit brackish marshes and lagoons in southern Iran and Iraq (De Grave and Ghane 2006; Salman et al. 2006; Gorgin and Sudagar 2008), and has been recorded in Dagestan and downstream of the River Don (Afanasyev et al. 2020; Zhivoglyadova et al. 2021). In Europe, the species has been registered in Bulgaria, Moldova, Romania and Ukraine (Zhmud et al. 2022; Surugiu 2022; Bushuiev et al. 2023; Kutsarov et al. 2023; Munjiu et al. 2023). Entrepreneurs in Ukraine are now considering the potential of Oriental river prawns as an important fisheries resource (Bushuiev et al. 2023). Before this can be put into effect, however, effective monitoring strategies will be needed to prevent the spread of this invasive species and to maintain balanced aquatic ecosystems (Jones et al. 2021). This is particularly important as Oriental river prawns have been shown to actively regulate populations of other aquatic organisms (Thornber et al. 2020).

Therefore the aim of this study is to determine key morphological and morphometric features useful for distinguishing Oriental river prawn populations in different Ukrainian waterbody types, and to describe any seasonal and/or regional behavioural differences between populations.



Figure 1. Map of sampling localities of the Oriental river prawn (*Macrobrachium nipponense*) in southwestern Ukraine. 1 – Sukhyi Lyman; 2 – City of Vylkove; 3 – City of Kiliia

Table 1. Number of prawn individuals separated by sex and localities sampled in 2022–2023

Sampling site	Latitude	Longitude	Date	Males	Females	Total	
Sukhyi Lyman	46.393345	30.632992	06/2023	21	14	35	
Ochakiv Branch	45.392936	29.612353	08/2023	11	3	14	
Bazarchuk	45.416121	29.550168	02, 06–08/2023	62	53	115	
City of Vylkove	45.410767	29.606138	07, 10/2023	48	16	64	
	45.467267	29.595733					
	Kiliia Branch	45.408139	29.554472	07–08/2022	19	39	71
	Solomonov Branch	45.426667	29.543700	06–12/2022 03–05, 07–09, 12/2023	170	84	455
City of Kiliia	Kyslytsky Branch	45.448750	29.235250	07/2022	15	0	15
		08/2023	15	13	28		

Materials and methods

Oriental river prawns were sampled from three localities, e.g. the Sukhyi Lyman Estuary, Danube delta (city of Vylkove) and Kyslytsky Branch (city of Kiliia), between June 2022 and December 2023 using 5–7 mm mesh fyke-nets (Figure 1; Table 1). In the city of Vylkove the sampling was provided from five different sites, including of Ochakiv Branch, Bazarchuk, Vylkove canals, Kiliia Branch, Solomonov Branch (see Table 1). In total, 983 prawns were collected and analysed, comprising 473 males, 296 females and 115 juveniles, with 99 individuals of unidentified sex.

For all trapped individuals, we measured length from the anterior rostrum end to the posterior telson end (L , mm) and weight (W , g). The ovigerous females were weighted together with clutch. In addition, we measured the length of both right (L_{pr} , mm) and left (L_{pl} , mm) 2nd pereopods, and counted the number of dorsal (D_d) and ventral (D_v) rostrum spines (denticles). Finally,

we calculated left and right pereopod length indices, and their ratio, for each prawn using the equations:

- 1) Index of right pereopod length (I_{pr}).

$$I_{pr} = L_{pr} / L$$

where L is total body length and L_{pr} is right pereopod length.

- 2) Index of left pereopod length (I_{pl}).

$$I_{pl} = L_{pl} / L,$$

where L is total body length and L_{pl} is left pereopod length.

- 3) Ratio of right to left pereopod length (I_{RL}).

$$I_{RL} = L_{pr} / L_{pl},$$

where L_{pr} and L_{pl} are the lengths of right and left pereopods, respectively.

To assess dependence of weight to length, we applied the linear relationship:

$$W = a \times L^b,$$

where W and L are the weight and length of the species, a is the intercept of the linear equation in log-transformed coordinates for the vertical axis and b is the growth coefficient (see Froese 2006). To obtain coefficients a and b , the non-linear relationship was linearised through log-transformation (Feng et al. 2014) and regression analysis used to assess correlations between the data obtained (Quinn and Keough 2002). Weight and length data for males and females were transformed using the natural logarithm function. The significance of the Pearson correlation coefficient was checked with the corresponding t-test. The data thus obtained was used to compare males and females from different seasons (juveniles and individuals with unclear sex not tested). For seasonal analyses, all samples collected around the City of Vylkove were treated as coming from the same locality. Comparative analyses of data from Sukhyi Lyman, Vylkove and Kiliia was only possible for summer.

The Kruskal-Wallis test was applied to determine whether there was a significant difference between the medians of three or more independent groups (Miller 1997; Ostertagová et al. 2014). Cluster analysis was then conducted on the samples using Ward's linkage method (Everitt et al. 2011). All statistical computations and visualisations were conducted in the R software environment (R Core Team 2021).

Results

The data from Vylkove indicated that, overall, male prawns tended to slightly dominate in the overall (annual) catch (mean 54.79%), but especially during the spawning period (June–Sept), while females dominated the catch during the feeding period (March; Figure 2). Apart from June, when only two females

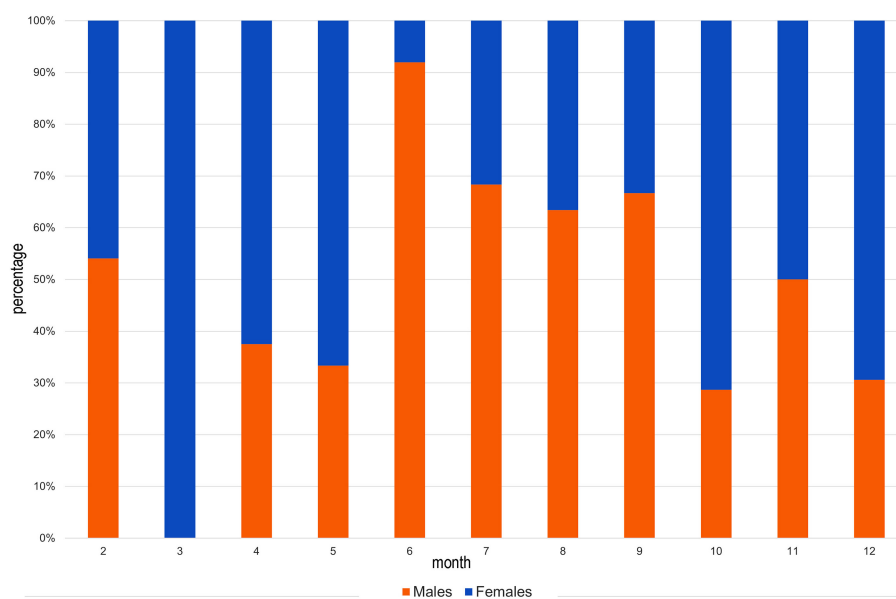


Figure 2. Variation of Oriental river prawn male/female ratio (%) in the Danube delta in Vylkove, Ukraine

were caught, both of which were ovigerous, the data indicated a clear peak in ovigerous females in August (80%). At Sukhyi Lyman, which was only sampled in June, 85.7% of females were ovigerous (12 of 14), while at Kiliia, only sampled in August, 61.54% were ovigerous (8 of 13; see Supplementary material Table S1).

Overall, variability in L , W , L_{pr} , L_{pl} , I_{pr} and I_{pl} tended to be higher in males than females, with highest variability in L and W observed in winter and autumn, and summer and autumn for L_{pr} , L_{pl} , I_{pr} and I_{pl} (Figure 3). There was no significant difference ($p > 0.05$) between I_{pr} and I_{pl} for males or females. Both D_d and D_v parameters have no significant difference between sexes or seasons, though male D_d tended to be more variable in spring and summer, and female D_d in summer and autumn (see Table S2, Figures S1, S2, S4).

During summer, the same parameters suggested prawns from the brackish water at Sukhyi Lyman were smaller, though not significantly different from those caught in the Danube basin ($p > 0.05$; Table 2; Figure 4). While there was no significant difference ($p > 0.05$) in D_d and D_v by locality, D_d was more variable at Vylkove for both males and females (Table 2; see Figures S5–S8).

Length-weight relationships were strongly significant for both male and female Oriental river prawns at Vylkove (Pearson coefficients: male = 0.989, female 0.956; $p < 0.001$; Figures 5A, B), and for summer catches at all three sites (Pearson coefficients: male = 0.988, female = 0.960, $p < 0.001$; Figures 5C, D).

Kruskal-Wallis tests indicated significant seasonal differences in L , W , L_{pr} , L_{pl} , I_{pr} and I_{pl} in males and L , W , L_{pr} and I_{pl} in females, as well as significant differences between three localities, with males differing in L , W , L_{pr} , L_{pl} , I_{pr}

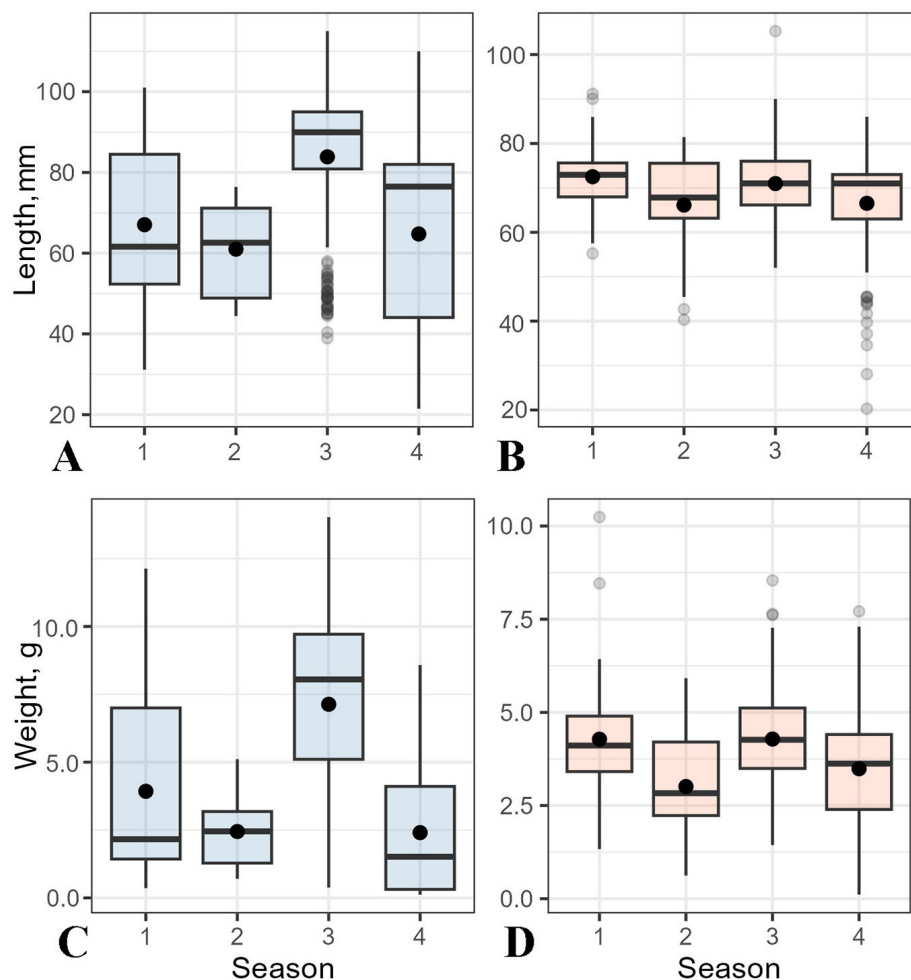


Figure 3. Seasonal variation of lengths (A, B) and weight (C, D) parameters of males (A, C) and females (B, D) from Vylkove (seasons: 1 – winter, 2 – spring, 3 – summer, 4 – autumn)

Table 2. Morphological parameters of male and female individuals of prawns collected in the summer season. L – total length (mm), W – weight (g), L_{pr} – length of right pereiopod (mm), L_{pl} – length of left pereiopod (mm)

	Sukhyi Lyman		Vylkove		Kiliia	
	♂ (n = 15)	♀ (n = 14)	♂ (n = 224)	♀ (n = 102)	♂ (n = 30)	♀ (n = 13)
L	57.92 ± 13.73	47.33 ± 7.56	83.87 ± 17.52	70.97 ± 8.41	95.18 ± 8.49	81.06 ± 6.53
W	24.05 ± 15.9	11.32 ± 6.58	71.33 ± 36.23	42.84 ± 13.67	94.16 ± 20.65	61.88 ± 19.38
L_{pr}	57.21 ± 24.18	27.98 ± 9.8	98.36 ± 35.17	44.16 ± 9.92	114.68 ± 24.02	49.81 ± 5.39
L_{pl}	53.87 ± 22.22	27 ± 5.71	99.13 ± 36.57	44.24 ± 10.46	114.12 ± 18.77	51.15 ± 5.74
Ir	9.41 ± 2.51	5.95 ± 1.28	11.12 ± 3.04	6.11 ± 1.02	12.03 ± 2.19	6.1 ± 0.45
Il	8.92 ± 2.47	5.79 ± 0.82	11.2 ± 3.1	6.15 ± 1.14	12.1 ± 1.57	6.24 ± 0.42
Irl	1.07 ± 0.18	1.02 ± 0.31	1.02 ± 0.40	1.00 ± 0.14	0.986 ± 0.16	0.99 ± 0.05
Dd	13.27 ± 0.70	13.36 ± 0.63	13.15 ± 1.40	12.95 ± 1.24	13.0 ± 0.98	13.0 ± 1.0
Dv	3.0 ± 0.38	2.86 ± 0.53	3.22 ± 0.75	3.06 ± 0.52	3.2 ± 0.41	3.31 ± 0.48

and I_{RL} and females in L, W, L_{pr} , I_{pl} and D_v (see Table S3). Seasonal cluster analysis (Figure 6) showed males to be similar in all seasons except for summer, but showed two clusters for females, i.e. a winter-autumn group and a spring-summer group (Figure 6).

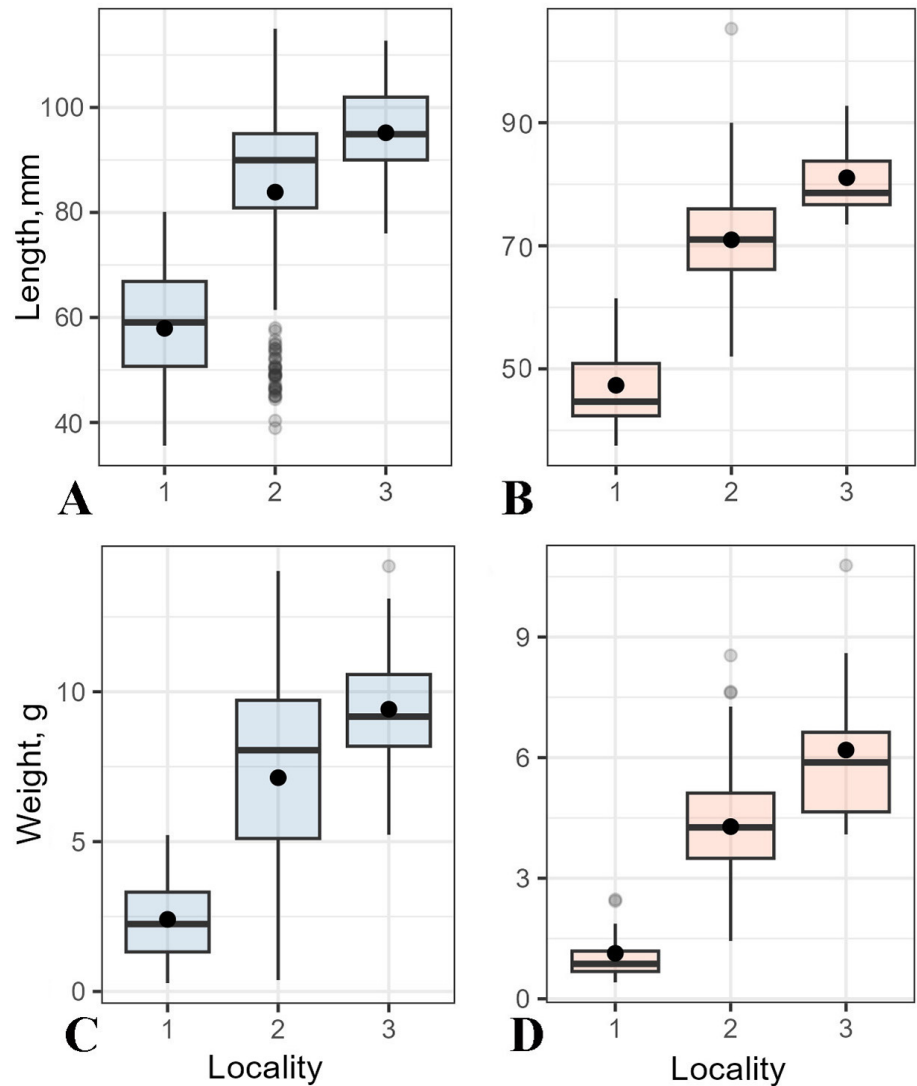


Figure 4. Length (A, B) and weight (C, D) parameters of males (A, C) and females (B, D) from different localities (Locality: 1 – Sukhyi Lyman, 2 – Vylkove, 3 – Kiliia)

Discussion

In this study we presented the population characteristics of the invasive Oriental river prawn populations from Ukraine for the first time. This also serves as the first report of population characteristics of the invasive Oriental river prawn in Europe. Characteristics of linear growth of *M. nipponense* in the new area turned out to be similar to those in their native area of Myanmar (Aye 2020). A stable weight-length dependence was observed in both sexes, with an isometric type of growth characteristic of this species. The determination coefficient (R^2) values in males were slightly higher than in females probably due to the greater variability in the mass of females, which can be attributed to the presence of eggs on their pleopods or under the carapace (Figure 5). In both cases, the slightly lower figures for females are likely to reflect variation due to pre- and post-spawning condition. It is also worth noting that the length-weight relationship in female *M. nipponense* from the Danube River was higher (R^2 0.95–0.96) than in the natural habitat

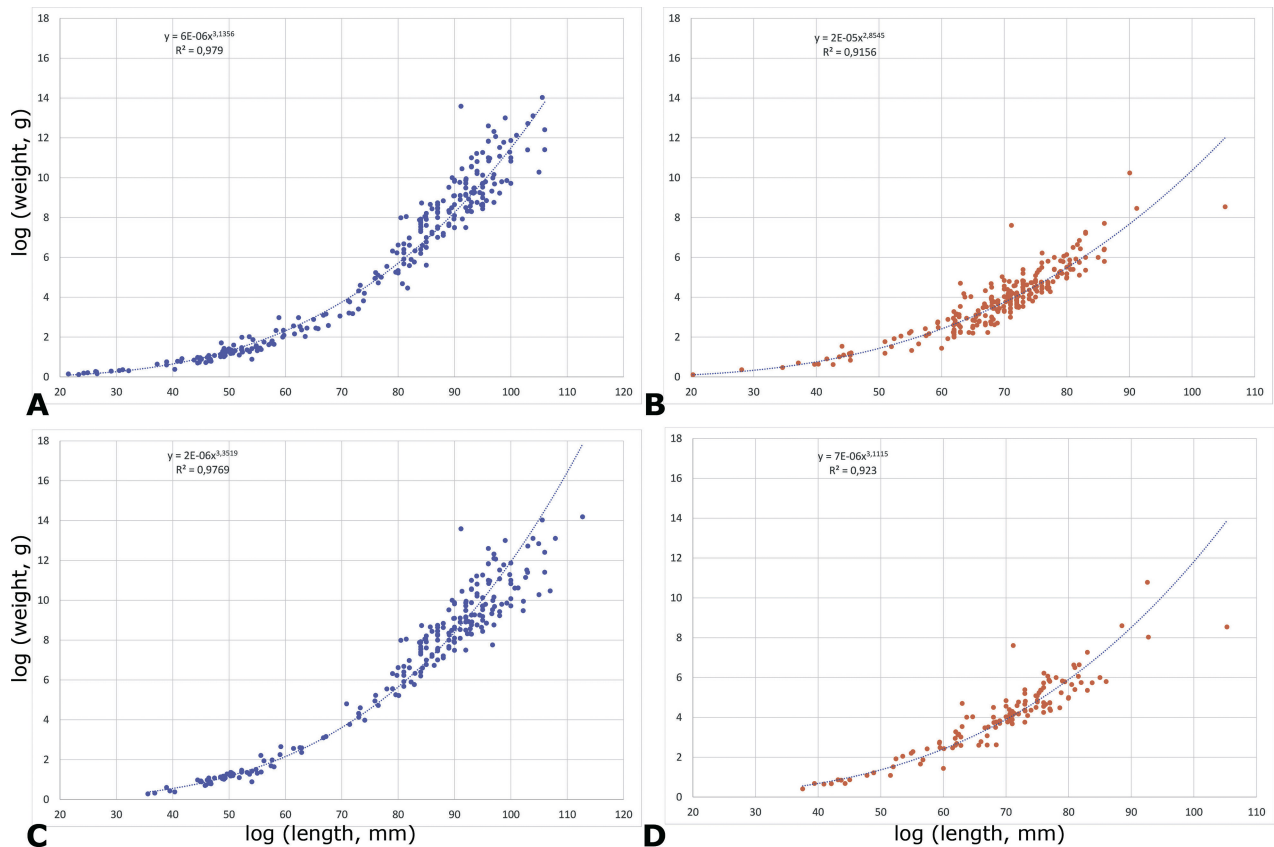


Figure 5. Linear regressions between weights and lengths for male (A, C) and female (B, D) individuals in different seasons in Vylkove (A, B) and in different localities in summer period (C, D)

water bodies (R^2 0.64–0.83) (Aye 2020). This phenomenon is likely because the prawn spawning period in Ukrainian waters occurs in a more compressed timeframe due to seasonal temperature fluctuations. Therefore, it can be assumed that the maturation of gonads in most of females in the study area happens more synchronously, leading to less variability in their mass characteristics than in native range (Aye and Sein 2012; Aye 2020).

The correlation between length and weight was higher here than in the tropical part of the native range (in Myanmar). In the tropics, weight growth with length is not as uniform as it is in Ukraine. The reason may lie in the climate: in Ukraine there is a dormancy period (winter) when the prawns do not grow. In Myanmar, seasonality is associated with rains, and the temperature is stable, so prawn growth depends on factors other than seasons, particularly the availability of food (Aye and Sein 2012; Aye 2020). In the Danube River, the food availability in winter and summer periods have more inexpressive seasonal dynamics, than in tropics (Goncharov 2005; Dzhurtubayev et al. 2013; Danube Commission 2024).

The current study showed that the most stable morphological feature of the Oriental river prawn in SW Ukraine are the denticle numbers, both dorsal and ventral. Variation in both D_d (11–19 denticles) and D_v (2–5 denticles) was within parameters typical for the species. This feature could be excluded in the population analyses, because it is stable for the sampled localities

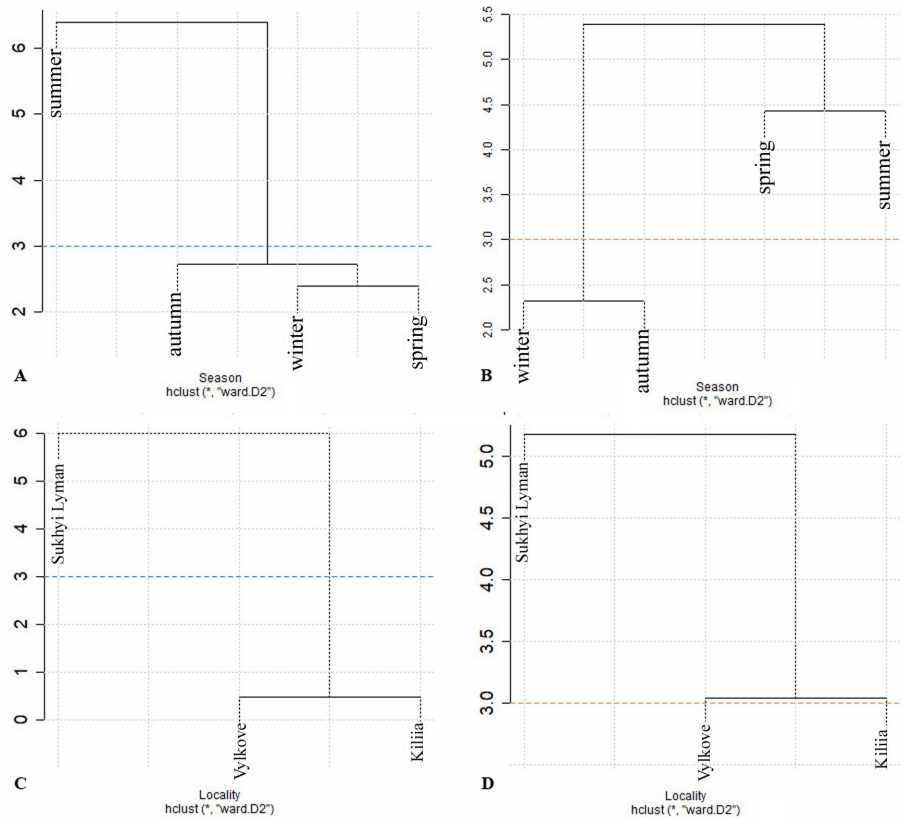


Figure 6. Hierarchical clustering dendrogram of male (A, C) and female (B, D) individuals by seasons (A, B) and localities (C, D)

and seasons. The rest of the parameters are useful for population tagging and can also reflect seasonal changes. The body length and weight, as well as 2nd pereopod lengths and related indices are helpful for group discrimination and seasonal variability.

Because the differences between left and right pereopod length are not observed, only one pereopod could be used in the further studies. We registered the differences in the 2nd pereopods lengths among seasons. We note as well the high variability of the 2nd pereopods lengths and related indices in males in the summer and autumn periods (see Figure 4; Figure S5). In river prawns of the genus *Macrobrachium*, the size of male 2nd pereopods is an important parameter for indicating their fighting ability, which is noted for the Giant river prawn (*Macrobrachium rosenbergii* (De Man, 1879)) (Barki et al. 1992). The reproductive biology not only includes the production of hatchlings released by the species, but also the activity and behaviour shown by males and females during mating, fecundity, breeding season, and maturity age. The guarding of females approaching a limited period of sexual receptivity is a common mating tactic of males. Typical mate guarding behaviours have been observed in many decapod males (Bauer 1976; Ridley 1983). The dominant males are very aggressive in stage of pre-mating activities, vigorously chasing the other males who came closer to female. Sometimes a powerful attack can lead to lesions in the rostrum or pereopods of recessive

males (Sarkar et al. 2012). As a result of aggressive behaviour during the spawning period, decapod males may lose pereopods (Barki et al. 1997). Variability in the size of second pereopods during the summer period can be the result of the loss of pereopods due to aggressive behaviour during the spawning period (Barki et al. 1991). In the Ukrainian populations of the Oriental river prawn this variance likely stems from behavioural differences during the breeding season. It appears that females, in preparation for egg-laying on pleopods in June, tend to seek shelter and move less, a behaviour that persists until the end of spawning (Ismael and New 2000). Conversely, they become more active in feeding during autumn, likely resulting in increased catch rates in fishing gear (see Figure 2).

In Ukraine, the males are predominant in the samples, which agrees with the data from the native range. For example, in Myanmar males comprised 56.73% of the population (Aye and Sein 2012). Because the climate in Myanmar is tropical and differs significantly from the temperate climate in SW Ukraine, we can say that this feature is stable for different populations. A skewed sex ratio can be caused by different mortality rates between sexes and different factors, such as distribution, ovigerous females, sex reversal, temperature, or crowding (Kim 2005). The spawning season in Ukraine was observed from June till October, when the ovigerous females were recorded, with the maximum in August (see Table S1). It differs both from tropical and temperate parts of the native area of this prawn. Thus, in Myanmar the spawning was observed over the whole year with maximum number of ovigerous females in January and minimum in June (Aye 2020). In Japan, the climax of the breeding season is July–August (Mashiko 2000). In China in monoculture in the subtropical climate the prawn spawning was observed in late April–June, but the berried females were observed mainly in May–June (Hongtuo and Jin 2018). In general, in the native area the water temperature necessary for the spawning might not be lower than 15 °C (Kutty and Weimin 2010). In the Danube delta the warmest months are July and August, when the water temperature is on the level of 24–26 °C (Danube Commission 2024). The water temperature higher than 15 °C is observed since April–May till October, which is similar to the temperate region of the native range of the prawn, for example in lakes Biwa and Kasumigaura in Japan (Fukushima et al. 2019, 2022). Nevertheless, the spawning period is much longer in Ukraine, which is one of the possible triggers to establish the stable population of this subtropical invasive species.

Research results from the native area in different types of water bodies in Japan showed opposite results to our data (Mashiko 2000). In Japan, the body size of females is much smaller in freshwater localities, than in brackish water, e.g. individuals up to 60 mm length were registered in brackish lakes Kojima and Shinji, but in rivers Sagami, Aza, lakes Naganuma and Biwa they were up to 40–50 mm (Mashiko 2000). In Ukraine, the females of same

size observed in the brackish Sukhyi Lyman, but in freshwater localities in the Danube delta they are much larger, having average sizes of 70–80 mm. In the native area the biogenic content in freshwater is much lower, and estuaries and lagoons are places of higher production. In Ukraine there are high productivity in fresh water, especially in deltaic environments (Goncharov 2005; Dzhurtubayev et al. 2013). This could lead to significantly larger sizes of prawns in the invaded range in Ukraine, than in the native one. Due to the larger size and possible growth rates in the Danube delta than in the brackish estuary, we can conclude that brackish water bodies in Ukraine have lower potential for fisheries and prawn farming of the Oriental river prawn.

The spread of the Oriental river prawn in Ukrainian water bodies requires the development of management strategies taking into account both the commercial importance of this species potential of this species and its possible negative impact on native ecosystems (Bushuiev et al. 2023; Nekrasova et al. 2024). The successfully established population in the SW Ukraine requires the complex risk assessment of this non-indigenous species to understand the possible impact on the ecosystem of the Danube River.

Author's contribution

Anastasiia Lepkha provided the sex identification and measurements of the sampled prawns, also analysed the data and prepared the first draft of the manuscript. The critical comments and significant modification of the results and discussion was provided by Sergii Bushuiev. Statistical analysis and explanation of its results provided by Bohdan Hulak and Yevhen Leonchuk. The main ideas of the manuscript and its general structure were formulated by Yuriy Kvach. The final approval of the manuscript was conducted by all authors.

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Supplementary material

The following supplementary material is available for this article:

Table S1. Number and percentage of ovigerous females of the Oriental river prawn by month in different water bodies of Ukraine.

Table S2. Morphological parameters of male and female individuals of prawns from Vylkove by season.

Table S3. Kruskal-Wallis test for total length (L, mm), weight (W, g), lengths of 2nd pereopods, right (L_{pr}, mm) and left (L_{pl}, mm), Index of total length to right (I_{pr}) and left (I_{pl}) pereopod, ration of right/left pereopods (I_{RL}), number of dorsal (D_d) and ventral (D_v) denticles of the Oriental river prawn individuals differences by season and by locality. Significant differences are in bold

Figure S1. Seasonal variation of right (L_{pr}; A, B) and left (L_{pl}; C, D) pereopod lengths parameters of males (A, C) and females (B, D) from Vylkove.

Figure S2. Seasonal variation of the Index of right (I_{right}; A, B) and left (I_{left}; C, D) pereopod lengths parameters of males (A, C) and females (B, D) from Vylkove.

Figure S3. Seasonal variation of the Ratio of right to left (I_{RL}) pereopod length parameters of males (A) and females (B) from Vylkove.

Figure S4. Seasonal variation of dorsal (A, B) and ventral (C, D) denticles numbers of males (A, C) and females (B, D) from Vylkove.

Figure S5. Parameters of right (L_{pr}; A, B) and left (L_{pl}; C, D) pereopod length of males (A, C) and females (B, D) from different localities.

Figure S6. Index of right (I_{right}; A, B) and left (I_{left}; C, D) pereopod lengths parameters of males (A, C) and females (B, D) from different localities.

Figure S7. Ratio of right to left (I_{RL}) pereopod length parameters of males (A) and females (B) from different localities.

Figure S8. Variation of dorsal (A, B) and ventral (C, D) teeth numbers of males (A, C) and females (B, D) by different localities.

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