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THE SPATIO-TEMPORAL DISTRIBUTION OF THE FRESHWATER CRUSTACEAN *ASELLUS AQUATICUS* LINNAEUS, 1758, IN THE DANUBE DELTA

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ABSTRACT

Asellus aquaticus Linnaeus, 1758 is the widespread and common Palearctic freshwater isopod, feeding on detritus. This crustacean, probably, has its ancient evolutionary origins around Siberia and has spread from there to Western Europe and North America. Commonly associated with a temperate climate, it has been recorded as far south as the Mediterranean, and as far north as Scandinavia. The species is primarily associated with the b-mesosaprobic waters of the palearctic realm i.e., high concentrations of dissolved oxygen, low oxygen consumption rate and significant mineralization of organic materials with end-products such as nitrates. Furthermore, it is considered an effective bioindicator since it is recognized for its high tolerance to organic pollution. The data obtained within this study were used to analyze the internal dispersal of *A. aquaticus* along the main branches of the Danube Delta (Chilia, Sulina and Sf. Gheorghe). For this purpose, 167 sediment samples were collected from 126 sampling sites between 2019 and 2020. Overall, according to obtained data, the individuals of the isopod *A. aquaticus* were identified only in 12 stations distributed throughout the investigated areas. The results may provide a simple biological tool which could be used to generally monitor water quality with more naturally occurring organisms. As well, it may contribute to get a thorough understanding of the ecology of freshwater systems. This assessment provides a baseline for further distribution studies of *A. aquaticus* and the details of his habitat requirements. Acquired data should result in more effective involvement of this species in a regional/national system of freshwater ecological status assessment.

Keywords: Danube Delta, freshwater crustacean, *Asellus aquaticus*, habitat distribution

INTRODUCTION

The water louse *Asellus aquaticus* (Linnaeus, 1758) is a common Palearctic freshwater isopod with its ancient evolutionary origins probably around Siberia and it is the most common and widespread European species of the Asellidae family from Siberia to western Europe and North America - commonly associated with a temperate climate [1]. It has been recorded as far south as the Mediterranean, and as far north as Scandinavia [2] in a variety of freshwater to brackish habitats. The species is primarily associated with the b-mesosaprobic waters of the palearctic, which is an area defined by high dissolved oxygen concentrations, low oxygen consumption and significant

mineralization of organic materials with end-products such as nitrates. *A. aquaticus* is abundant across much of its range, and its distribution has rarely been influenced by anthropogenic pressures with a tolerance to poor water quality and organic pollution [2]. However, it is sensitive to sustained high temperatures with negative effects on rates of growth, survival and reproduction. While males of the species are generally larger than females, it was suggested that the size of *A. aquaticus* is related to the environment in which it lives, as individuals from clean water sites tend to be larger than those from polluted sites [2]. Similarly, smaller individuals may be present in locations with higher temperature readings. *A. aquaticus* is known to feed primarily on decaying vegetation, microscopic algae and small invertebrates. It can also live on fungi and bacteria associated with detritus and it is also known to feed on periphyton [3]. While in Southern Scandinavia, *A. aquaticus* is semelparous (reproduce only once before death) and has one or two generations per year, populations in other regions are known to be iteroparous and reproduce more than once [2]. *A. aquaticus* occurs in abundance in habitats of both *Phragmites australis* and *Chara tomentosa*. However, in habitats of macrophytes which die off during the winter the density of *A. aquaticus* is low, probably due to the slow colonization. Migration is not well studied in *A. aquaticus* and there is little information on how movement is related to individual variation in traits such as body size and length of legs. In other arthropods, there is evidence for a connection between relative leg length and movement speed and the same has been found in other taxa during range expansion. Furthermore, there is evidence of fluctuating asymmetry in land living isopods on the ischium and merus, which can arise from either environmental or genetic stress [1]. The presence and the distribution of *A. aquaticus* in Romania has not been documented properly yet neither its range area in the Danube River or Danube Delta. Therefore, data collection is required to assess its abundance and determine its habitats preferences. From previous studies, *A. aquaticus* is found in abundance throughout Europe and in both brackish and freshwater habitats with a high tolerance to poor water quality. Hence, *A. aquaticus* is expected to be largely distributed in the Danube Delta. This study will aim at providing a first record of the distribution of *A. aquaticus* and its abundance in the branches of the Danube Delta in order to determine its habitat and water quality preferences for further research on its ecology.

MATERIALS AND METHODS

In order to analyze the dispersal of *Asellus aquaticus* (Linnaeus, 1758), 167 samples were collected from 126 stations from the main branches with rectified meanders and with the adjacent small channels from Chilia, Sulina and Sf. Gheorghe on the Danube Delta in 2019-2020. From the total of samples, the isopod *A. aquaticus* was only found in 12 stations (DD19-07 Tătaru Arm; DD19-22 Tulcea Arm – Mm 42,5; DD19-26 Tulcea Arm - Mm 35; DD19-32 Ceatal Sf. Gheorghe - Km 108; DD19-36 Sf. Gheorghe Arm – Km 51 + 700 m – in Perivolovca Channel 500 m; DD19-37 Sf. Gheorghe Arm – Km 53; DD20-50 Caraorman Channel; DD20-130 M. Sackhalin- Ciotic Channel; PCB-09-01 Busurca Channel; P10-SU05-08 Sulina Arm (Meander); PM-09-21B Old Danube (Meander); L06 Gorgostel Lake). The macrozoobenthic sampling is part of the monitoring network for the study of the biodiversity and for the assessment of biological quality of the main branches with rectified meanders from Danube Delta (Fig. 1). Additional benthic organisms found in the samples were studied and analyzed.

Those groups were reported in the samples but are not the subject of this study, only in the qualitative evaluation.

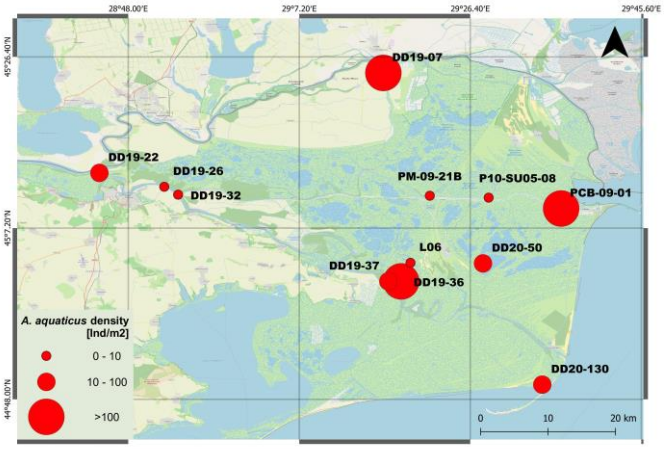

Figure 1. Map of the study area and the abundance of *A. aquaticus* individuals per sample

Sample processing and analysis

The sampling strategy [4], took into consideration the heterogeneity of substrate, and employed the multihabitat technique (a modified version of AQEM method used for water bodies monitoring in Romania – [5]). The multi-habitat sampling method (per sq. meter) was developed in compliance with type-specific river conditions in Romania and is implemented by the "Romanian Waters" National Administration. According to this technique, the habitat types and the proportion between them have been established. Subsequently, a fiche of habitats has been filled in. All habitats with a coverage of more than 5% were noted and classified according to [5]. R/V ISTROS and a small boat (in custody of NIRD GeoEcoMar) were used for measurements and for sampling the selected sites. Three quantitative replicas from each microhabitat were collected considering that this number is sufficient for estimating the diversity and density of invertebrates for every station from the Lower Danube River and Danube Delta. An average of three replicas of each station was used for the final assessment. 167 quantitative samples were collected by using two different Van Veen grabs. The abundance results (number of individuals) were expressed per unit surface (1 m^2) using a multiplication factor of 25.2 for the small Van Veen grab and 7.4 for the big Van Veen grab [4]. On board of the boats, the samples were washed through 250 and 125 μm sieves or through a limnological net with 125 μm mesh size in order to remove the excessive sediment particles and keep the macrofauna. A mixed solution of Rose Bengal and buffered formaldehyde 4% was used for staining and for the preservation of benthic organisms until subsequent analysis. In the laboratory, the samples were sorted and the organisms were identified at the lowest taxonomical level possible using a Carl Zeiss SteREO Discovery V8 microscope and a Carl Zeiss Axiostar microscope. The taxonomic identification was done [6]. The sample processing and analysis were carried out [7]. Granulometric analyses were performed by the diffractometric method with the 130 "Mastersizer 2000E Ver.5.20" laser granulometric, Malvern. Separation of granulometric classes (sand, silt, clay) and fractions within each class were defined according to the Udden-Wentworth logarithmic scale completed by detailing three fractions at the 1φ interval in the clay field as follows: grains non perceptible by eye

(<0.125 mm – silt clay), grains perceptible by eye (0.125-0.5 mm - fine sand), coarse sand (0.5-2 mm), gravel (2-16 mm), pebble (16-34 mm), cobble (34-256 mm), and boulder (>256 mm) [8]. For particle size analysis, the granulometry composition, the presence of detritus, aquatic vegetation and/or mollusk shells (debris), also recorded in samples, were not taken into account (Table 1 and Fig. 2).

Figure 2. Different substrate types from the main branches of the Danube Delta

DNA barcoding

Genetic analyses were performed to confirm the correct identification of the species as Asellus aquaticus using the barcode gene COI (Cytochrome c oxidase subunit I). During the sieving process, few organisms were selected, photographed and stored in Tris-EDTA pH 8 buffer at -20˚C until analysis. The DNA was extracted from the collected specimens using the DNeasy Blood & Tissue Kit (Qiagen, USA) and quantified using the BioDrop spectrophotometer. The fragments of the COI gene were amplified by PCR using the Mastercycler ProS System (Eppendorf, Austria) with the invertebrate primers LCO1490and HCO2198 [9]. The amplicons were analyzed by horizontal electrophoresis in 1% agarose gel (Cleaver scientific, Ltd) to verify the success of the amplification prior their purification using the QIAQUICK PCR PURIFICATION protocol (QIAGEN). The purified fragments were sent for Sanger type sequencing to Macrogen (Netherlands). The identification of the species was achieved by comparing the COI sequence with previously annotated homologous sequences in the GenBank database using NCBI BLASTn [10] completed in the nucleotide collection (nr/nt) database. The sequence was accordingly annotated and added to the GenBank database.

Statistical analysis

A multivariate principal component analysis (PCA) was applied to investigate the similarities between sites to estimate the conditions associated with levels of abundance. PCA would cluster sites on the two major axes of variation of the variables. The principal components (PCs) with eigenvalues greater than one were considered to be relevant [11]. Statistical analyses were performed in R v3.6.1 [12].

Studied species

Kingdom Animalia, Phylum Arthropoda, Subphylum Crustacea, Class Malacostraca, Order Isopoda, Family Asellidae, Genus Asellus, *Asellus aquaticus* (Linnaeus, 1758) Racovitza, 1919. This species can usually be distinguished from the frequent *Proasellus meridianus* by the pattern of pigmentation of the head, with two pale spots at the back separated by a central dark pigmented area. However, head pigmentation can vary and the two are frequently confused. The shape of the male first pereopod is diagnostic.

Maxillipeds with epipodites; biramate pleopod III, with large 2-articulated exopodite, long uropods with endo- and exopodites of equal length; the pleotelson has a tetrahedral contour with a sharp terminal tip; Marbled color on a gray background. This species exhibits sexual dimorphism, with males being larger, due to breeding with precopulatory mate-guarding, which is not uncommon in crustaceans. The maximum body length of males is up to 12 mm, while in females is up to 8 mm. *A. aquaticus* is abundant, occurring in a wide variety of water-bodies, including small urban garden ponds, ditches, lakes, canals and rivers. It can be found among water plants, under stones and submerged bits of dead wood, among the roots of riparian trees and on the stonework of bridges [6].

Figure 3. Specimen of *Asellus aquaticus* (Linnaeus, 1758) Racovitza, 1919

It is tolerant of organically polluted waters, high salinities, low pH and high metal concentrations. *A. aquaticus* is detritivore, feeding primarily on coarse particulate organic matter, fungi and algae. Autumn shed leaves constitute a large part of the diet. Thus, although it is present in a wide array of freshwater habitats including rivers, lakes, springs and subterranean waters, even brackish waters, it avoids oligotrophic freshwater habitats like fast-flowing mountain streams and mires [1]. The species commonly occurs in lower stretches of water courses with plenty of vegetation and trees. *A. aquaticus* is relatively tolerant to a range of pollutants and therefore has been used as a bioindicator [2]. According to the AQEM database, *A. aquaticus* is an alfa-mesosaprobic taxon, and a good indicator of organic pollution, with saprobic value of 2.80 and weighting factor 3 [5].

RESULTS AND DISCUSSION

The common Palearctic freshwater isopod, *A. aquaticus*, was found in 12 stations from 167 samples from 126 stations within the main branches with rectified meanders and with the adjacent small channels from Chilia, Sulina, Sf. Gheorghe on the Danube Delta. The highest densities of *A. aquaticus* was registered in the stations PCB-09-01 Busurca Channel, DD19-36 Sf. Gheorghe Arm – Km 51 + 700 m – in Perivolovca Channel 500 m and DD19-07 Tătaru Arm with 312.5 individuals/m^2, 201.6 individuals/m^2 and 151.2 individuals/m^2 respectively (Fig. 1 and Table 1). From the genetic analyses using the barcode gene COI, the sequence from our specimens have been identified as *A. aquaticus* with strong evidence given by 98% of the query cover matching the sequence. The genetic outcome proved the correct identification and confirmed the morphological description, thus reducing the potential confusion with *P. meridianus.* The sequence was added to the GenBank database and can be accessed with the accession number KJ676764.1. Our data show the *A. aquaticus* preference for sand

and clayey silt substrates, where the highest abundances were recorded, followed by stations characterized by sandy silt. In the other sediment types, this palearctic element has not recorded considerable values. However, we can notice an ecological plasticity in terms of sediment structure considering its high frequency of occurrence in the targeted areas.

Table 1. Stations, Coordinates, Particle size composition, TOC and Carbonates concentrations and abundance of *Asellus aquaticus* in the main branches from Danube Delta during the period 2019-2020

No Station	Coordinates Longitude	Latitude	Abund. Ind/m^2	TOC %	$CaCO_3$ %	Gravel + Shellfish %	Sand %	Silt %	Clay %	Shepard classification
DD19-07	45º24’36.1”	29º16’38.6”	151.2	0.395	11.685	0.00	28.05	56.93	15.03	Sandy silt
DD19-22	45º13’23.4”	28º44’45.7”	22.2	0.676	19.423	0.00	11.89	72.36	15.75	Clayey silt
DD19-26	45º11’50.1”	28º52’02.5”	7.4	0.542	10.186	34.52	50.88	11.39	3.21	Sandy gravel
DD19-32	45º10’57.0”	28º53’35.8”	7.4	0.741	9.060	0.00	24.23	59.09	16.68	Sandy silt
DD19-36	45º01’13.3”	29º18’38.5”	201.6	0.330	11.136	0.00	9.79	71.18	19.03	Clayey silt
DD19-37	45º01’13.6”	29º17’12.2”	25.2	0.052	7.979	0.00	99.17	0.67	0.16	Sand
DD20-50	45º03’14.4”	29º27’49.9”	50	0.084	7.883	-	-	-	-	Clayey silt
DD20-130	44º49’38.6”	29º34’29.2”	25	0.172	7.593	-	-	-	-	Clayey silt
PCB-09-01	45º 09’23,8"	29º36’36,4"	312.5	1.493	9.842	0.08	99.62	0.27	0.03	Sand
P10-SU05-08	45º10’36,9 "	29º28’28,0"	7.4	1.005	24.464	27.16	61.48	8.18	3.18	Sandy gravel
PM-09-21B	45º10’49,8"	29º21’51,6"	7.4	0.689	10.669	0.00	1.70	74.89	23.41	Clayey silt
L06	45º03’18,3"	29º19’41,3"	9	2.669	15.625	-	-	-	-	Clayey silt

In our research and in several studies, it has been demonstrated that *A. aquaticus* can be found on rocky substrate in associations with other organisms, specifically with Caspian mud shrimp, *Chelicorophium curvispinum* (G.O. Sars, 1895), *Dreissena polymorpha* (Pallas, 1771), the Ponto Caspian polychaeta *Hypania invalida* (Grube, 1860) and with Oligochaete species, mostly representatives of Tubificidae family. In addition to these species, trichopteran larvae have been identified, such as *Hydropsyche bulgaromanorum* (Malicky, 1977), Gastropod species like *Theodoxus fluviatilis* (Linnaeus, 1758), *Theodoxus danubialis* (Pfeiffer, 1828) or *Lithoglyphus naticoides* (C.Pfeiffer, 1828), chironomid larvae and sponges such as *Spongilla lacustris* (Linnaeus, 1758) and *Eunapius fragilis* (Leidy, 1851) [13]. In comparison with our study, the distribution of the *A. aquaticus* in Serbia (2007-2013) was found to be the dominant crustacean species in 26 localities situated in 24 watercourses in Serbia, including Danube River [14]. In the period 1999 -2003, it was reported in the Matita - Merhei lakes (Danube Delta) with densities of 1696 ind/m^2. It has also been reported in stagnant or slowly flowing waters of the Danube Delta, Razelm Sinoe, swamps of northern Moldavia, Basarabia and West region of the Country [15].

Table 2. Principal Component Analysis matrix with eigenvalues, percentage of the variance explained by each PC and their cumulative sum.

	PC.1	PC.2	PC.3	PC.4	PC.5	PC.6	PC.7	PC.8	PC.9
Eigenvalues	3.564	2.802	2.369	0.205	0.057	0.003	0	0	0
Variability (%)	39.604	31.133	26.318	2.277	0.632	0.036	0	0	0
Cumulative %	39.604	70.736	97.054	99.332	99.964	100	100	100	100

The limited number of stations with *A. aquaticus* present greatly reduced the statistical power of the analyses. No statistics model could be performed to identify the environmental conditions that would explain the abundance. More data is required to better assess the significance of each variable and data on samples without *A. aquaticus* need to be collected to obtain a complete model.

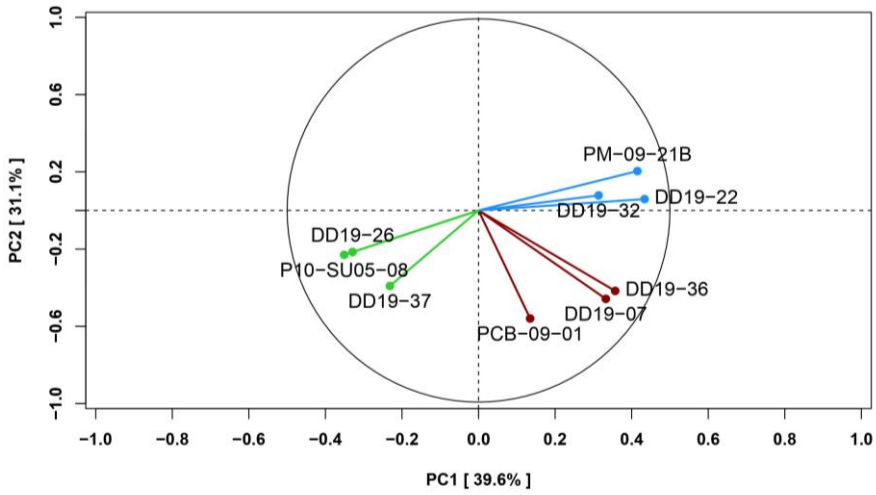

Figure 4. Principal Component Analysis on the species abundances and particle size of sediments, TOC and $CaCO_3$ concentrations

However, with 71% of the variance explained with the first two PCs, the PCA clustered the sites in three groups (Fig. 4). The sites with higher abundance were grouped together with a large influence from the abundance variable. The other two groups with sites of low abundance were split according to the sediment composition with sandy soil for DD19-26, P10-SU05-08 and DD19-37 and with silty soil for PM-09-21B, DD19-22 and DD19-32. From our data, there is no clear preference for any type of habitat neither for chemical water parameters where *A. aquaticus* would thrive with higher abundance. Even though *A. aquaticus* is tolerant to organic pollution and poor-quality water, its narrow distribution in the Danube Delta was unexpected especially since the Delta shows good quality water, stable temperature and little pollution [2]. This could be explained by other parameters not studied here or by impacts such as invasive species, heavy ship traffic or dredging. The distribution of *A. aquaticus* in the lower part of the Danube River requires additional research to reach a better evaluation of its ecology.

CONCLUSION

Overall, this study provided records on the presence of the isopod *Asellus aquaticus* in the Danube Delta and suggested a limited distribution without clear preferences of sediment composition or water quality contradicting observations from other areas. However, the exact reasons of these differences require additional investigation. Exploring other parameters could pinpoint the environmental factors that would dictate the distribution of *A. aquaticus* in the lower sector of the Danube River and whether the population is impacted by local stressors. In addition, this study may provide a simple biological tool which could be used to generally monitor water quality with more naturally occurring organisms. As well, may contribute to get a thorough understanding of the ecology of freshwater systems. This assessment provides a baseline for further distribution studies of *A. aquaticus* and the details of his habitat requirements. Acquired data should result in more effective involvement of this species in a regional/national system of freshwater ecological status assessment.

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