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Faculty of Biology and Geology

THE STUDY ON THE AQUATIC COMMUNITIES OF THE MALACOSTRACA (CRUSTACEA: MALACOSTRACA) FROM ANINA MOUNTAINS

– Thesis summary –

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CONTENT

Key words: amphipods, Aninei Mountains, decapods, ecology, isopods, malacostraca

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INTRODUCTION

The Malacostraca class (Arthropoda: Crustacea) groups medium and large crustaceans, that have the body well-defined into cephalothorax and abdomen. The class representatives of the sweet water fauna from our country belong to the Decapoda, Amphipoda and Isopoda orders, the first ones having only aquatic representatives, while the last one has also representatives adapted to a terrestrial life. (Radu and Radu 1967).

The Decapoda order presents a very varied morphology, the sweet water specimens being represented by crayfish with an unmistakable aspect (Băcescu 1967). The amphipods are a medium type of malacostraca, with an arched body. The sweet water species live in springs, streams, rivers, lakes, puddles, especially close to the shores, under rocks or aquatic plants (Popescu-Marinescu and Năstăsescu 2005), but there are also species that prefer ground waters, fountains, caves, springs (Cărăuşu *et al.* 1955). The isopods are crustaceans that were able to spread in all sort of life environments, ranging from the salty water of the seas, to low salinity areas and sweet water ones, from the low level of moisture of the undergrowth, of the moss or that of different objects that had fallen on the ground, to the dryness of open fields. (Radu 1983, Radu 1985).

From the point of view of the importance of the conservation of aquatic ecosystems, only the decapods have a different status (Souty-Grosset *et al.* 2005). *Austropotamobius torrentium* and *Astacus astacus* are species that the International Union for the Conservation of Nature labels as *vulnerable* (Sket 1996); the same species also enjoy the protection that was granted to them by the Bern Convention. The Habitats Directive (CEE 92/45), through Appendix 5, grants the status of *superior species* (UE code 1093*) to the decapod *Austropotamobius torrentium*. The national legislation requires a protection status through the O.U.G 57 from 2007, which is listed in Appendix 3 as an "*animal species whose conservation needs the depiction of the special conservation areas*", playing thus an important part in the designation of the Nature 2000 sites; at the same time they are listed in Appendix 4A as a "*species of community interest, as an animal species that requires a strict protection*". *Astacus astacus* is a species that has been included in *O.U.G.* 57 since 2007 in Appendix 5A as a "species of community interest, whose sampling and exploitation are part of the management measures."

The data that have been published concerning the malacostraca species from the area of the Anina Mountains are few and outdated. For decapods there are some references but many of the locations no longer corespond to reality. One of the first mentions about the *Austropotamobius torrentium* species from the Anina Mountains are from the Miniş river, from the Buhui lake and from other unspecified areas around the Anina Mountains (Entz 1912). In the volume Decapods of Romania Fauna the species is mentioned in more that one loaction. (Băcescu 1967). Bănărescu and

Oprescu (1971) mention the scattered presence of the species along some of the Nera afluents from the mountaineous area. The *Astacus astacus* species is mentioned in the specialised literature as being present in the Anina Mountains in the Buhui lake (together with *A. torrentium*), in the Miniş river (Băcescu 1967) or all along the Nera river (Bănărescu and Oprescu 1971). There are no mentions concerning the presence in the Anina Mountains of the *A. leptodactylus* species or of other decapod species. The most recent monography about the European crayfish "Atlas of crayfish in Europe" (Souty-Grosset *et al.* 2006), reflects through the lack of recent data a critical situation concerning the distribution of the three native species of crayfish in Romania.

Another important aspect for the conservation of the biodiversity is represented by the danger of the invasive species (Arbačiuskas *et al.* 2008, Grabowski *et al.* 2006, Grabowski *et al.* 2005). At the present moment there are in Europe ten species of sweet water decapods that have been intentionally introduced between the 19th and the 20th centuries (Ahem *et al.* 2008, Chucholl and Daudey 2008, Holdich and Pöckl 2007, Janský and Kautman 2007, Souty-Grosset *et al.* 2006, Henttonen and Huner 1999). On the other hand, the Danube corridor represents an upstream means of dissemination for some amphipod species, from the Balcan and Ponto-Caspian region, towards the western and northern part of Europe (Buřič *et al.* 2009, Grabowski *et al.* 2007a); this is the way in which the *Dikerogammarus villosus* has reached all the way to the alpine lake Lac du Bourget – France (Bacela *et al.* 2008, Grabowski *et al.* 2007b).

The investigated area represented by the Anina Mountains has a complex geology comprising mostly of lime stones (Bleahu and Rusu 1965), and the waters from the three hydrographic basins also flow through areas affected by human intervention. The effects of human activities that are not following a conservation policy generate dangers that may have a high impact on the aqautic biodiversity, since they produce reversible modifications for the water's phisio-chemical parametres which turn out to be irreversible for the structure of the aquatic fauna. (Petrovici 2009, Wang *et al.* 2007, Wang *et al.* 2006, Westman, in Alabaster 1985). The region is highly influenced by the impact of human intervantion that may take different forms: localities, mining, artificial dams; the region is under the supervision of the Administrations of two important National Parks: Semenic – Cheile Carașului and Cheile Nerei – Beușnița.

The Water Framework Directive, translated into the Romanian legislation through the Water Law 107 from 1996, completed by the Order of the Ministry of Environment and Water 161 from 2006, outlines clear rules concerning the admitted limits for each chemical substance that may affect the health of the fauna and of the habitat. The aquatic species tolerate differently the toxic effects (Füreder *et al.* 2003, Grandjean *et al.* 2003, Eversole and Seller 1996, Foster and Turner 1993). Pesticides have determined effects in the lowering of the abundance and diversity of aquatic organisms both vertebrate and invertebrate (Amy *et al.* 2008), while chemical fertilizers have an action in time, causing important disequilibrium (Bennett *et al.* 2008, McLaughlin and

Mineau 1995). The main toxic action nitrite have on aquatic animals is generated by the conversion of the pigments carrying oxygen in non-functional ways leading to hypoxia or even death (Camargo and Alonso 2006, Kozák *et al.* 2005, Jensen 2003, Jensen 1996, Tahon *et al.* 1988). The toxicity of the nitrate ions dissolved in water is considered irrelevant (Russo 1985, Camargo *et al.* 2005); however, just like in the case of nitrite, the main cause for the nitrate ion's toxicity is given by the conversion of the pigments carrying oxygen into inactive forms (Camargo and Alonso 2006), since nitrate may be in fact converted into nitrite inside the body of the animals (Cheng and Chen 2002). The concentration of soluble reactive phosphorus dissolved in surface waters vary in close connection with the variation of their concentration found in the soil (Smith *et al.* 2007). The chemical oxygen deficit is a highly utilised test for the indirect measuring of the organic concentration of the water (Eaton *et al.* 2005). The oxygen quantity that is dissolved into the water is essential for the well-being of aerobic organisms; its scarcity may lead to hypoxia and even mass (Camargo and Alonso 2006). The most important salts that are diluted in the mountain waters are those of calcium and magnesium. The calcium carbonate enters into the chemical composition of the crustaceans' crust. The hardness of the water has an effect on the toxicity of heavy metals that may be found in the water (Rathore and Khangarot 2002). Cyanides are chemical compounds extremely dangerous because they may discharge the free ion CN^-, which is highly toxic for virtually all life forms (Donato *et al.* 2007). The concentration of surfactants in the water depends on the interaction with the surfaces and on its hardness (Hu and Tuvell 1988).

Brief Characterization of the Anina Mountains

The Anina Mountains are part of the large mountainous unity of the Banat Mountains, lying at their western part. These mountains have had different names, part of them being attached to the Semenic Mountains or forming independent units, known as the Doman Mountains or the Caraş Mountains. Covering an area of almost 770 km^2, these mountains consist of an alternation of alpine summits and limestone plateaus being displayed on a NNE-SSV direction, having a medium length of approximately 50 km and a medium width of approximately 18 km (Sencu 1978).

Due to the very varied geology of the mountains, the hydrographic network from this region is extremely spectacular. In the limestone areas, the surface waters disappear into the underground to form breathtaking karstic scenery that is still active (Sencu 1978). Three major watercourses flow through this geographic unit: Bârzava, Caraș and Nera. There are also six artificial reservoirs in this area and one that is still to be built. In addition, there are also two natural lakes of karstic nature.

The characterization of the sampling stations for biological and chemical tests

<u>The sampling stations from the hydrographic basin Bârzava</u>

Along this river 17 sampling stations (figure 1) have been chosen for the study of the aquatic malacostraca, 6 of which are to be found on the main course of the river while the remaining 11 are on the river's tributaries. The stations on the main course have been placed between the area where the springs of the river are to be found and the area in which the river flows out of the mountains, as well as on the upstream and on the downstream of the reservoir lakes Văliug and Breazova, and on the area that marks the river's entry into the municipality Reșița.

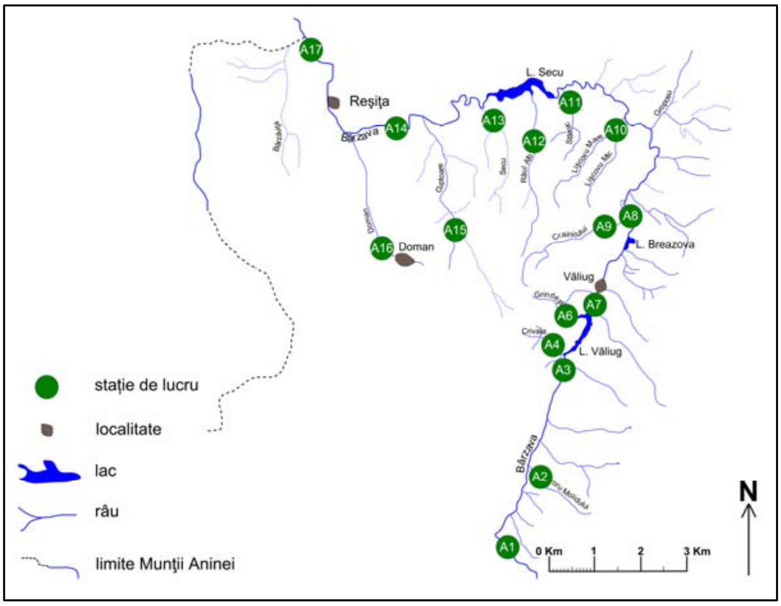


Figure 1: The sampling stations placed in the hydrographic basin of the river Bârzava (drawing done in accordance with the topographic map 1:50.000)

<u>The sampling stations from the hydrographic basin Caraş</u>

In this hydrographic basin a number of 22 sampling stations have been assigned for the study of the aquatic malacostraca (figure 2), 2 of which are place don the main course of the river and 20 are on the river's tributaries. The sampling stations on the main course have been placed near the river's springs and on the area in which the river flows out of the mountain.

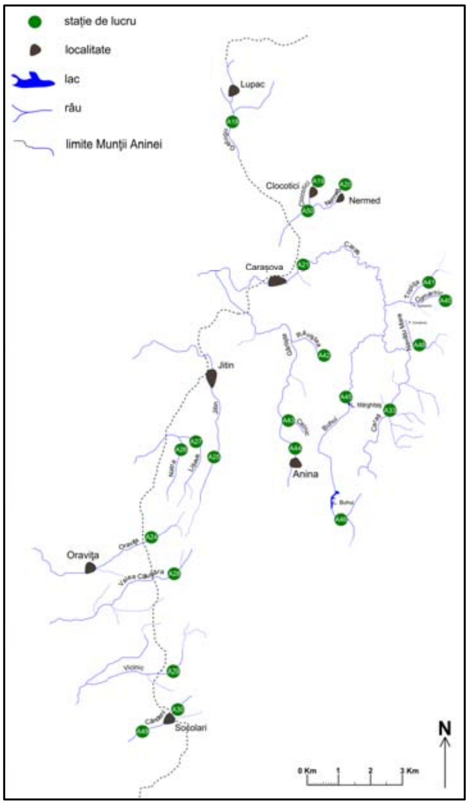


Figure 2: The sampling stations placed in the hydrographic basin of the river Caraş (drawing done in accordance with the topographic map 1:50.000)

The sampling stations from the hydrographic basin Nera

In this hydrographic basin a number of 13 sampling stations have been chosen (figure 3), out of which only one station is to be found on the main course of the river Nera, since this river runs through only a small area of the Anina Mountains.

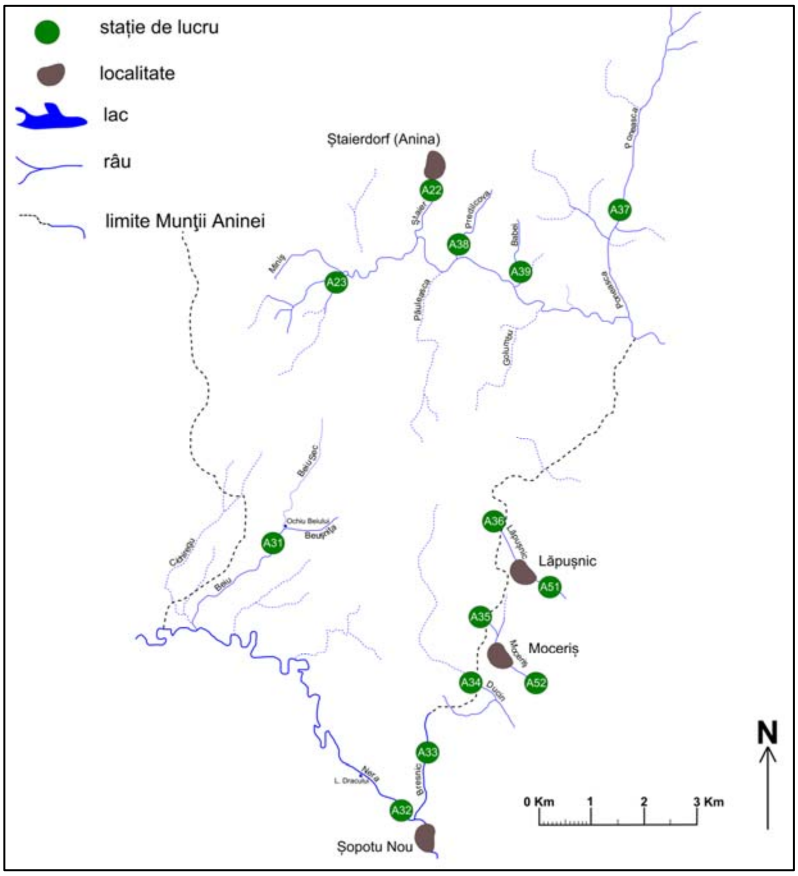


Figure 3: The sampling stations placed in the hydrographic basin of the river Nera (drawing done in accordance with the topographic map 1:50.000)

The quality of the surface waters from the Anina Mountains, according to the measured chemical factors

Taking into account the analyses that have been made in the surface running waters of the Anina Mountains during the summer of 2008 and 2009, there have been established the classes of waters that can be classified according to the level of nutrients, oxygen and other relevant chemical indicators, taking into consideration the Order of the Ministry of Environment and Water no. 161 from 2006.

Water classes from the hydrographic basin Bârzava

For the hydrographic basin of the river Bârzava, the measured data have been classified in figure 4. The values presented represent the average for the two years of study (2008 and 2009).

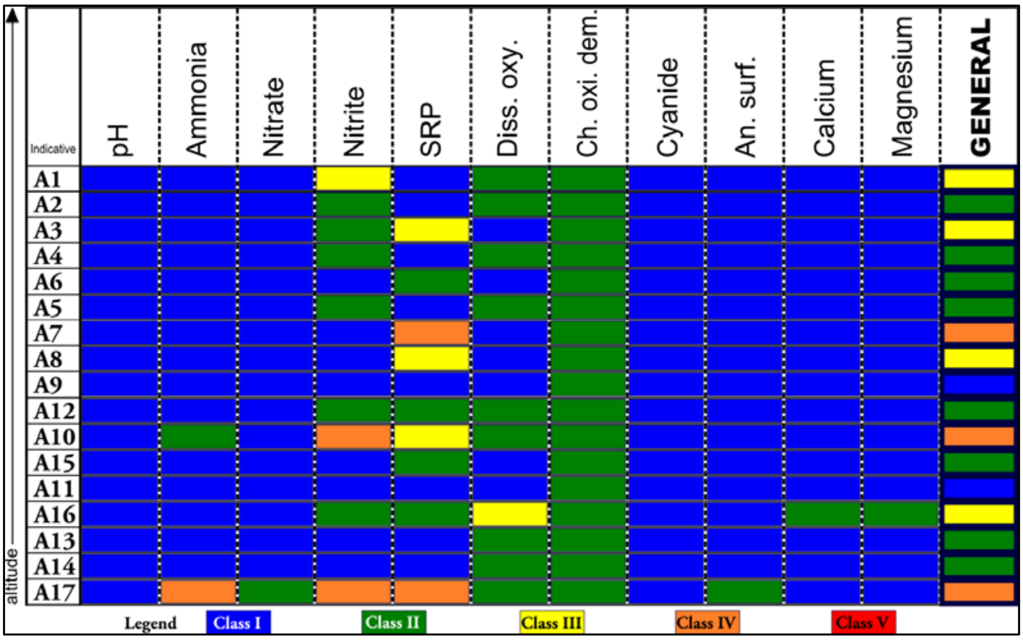


Figure 4: The water classes from the hydrographic basin Bârzava in accordance with the national system of classifying surface waters (O.M.M.G.A. 161/2006)

Indexes for the sampling stations: A1 – Bârzava izvoare, A2 – Molidului, A3 – Bârzava amonte, A4 – Crivaia, A5 – Văliug, A6 – Grindeşti, A7 – Bârzava aval, A8 – Bârzava aval, A9 – Crainicului, A10 – Liscov, A11 – Stârnic, A12 – Râul Alb, A13 – Secu, A14 – Bârzava amonte, A15 – Cuptoare, A16 – Doman, A17 – Bârzava aval

Water classes from the hydrographic basin Caraş

For the hydrographic basin of the river Caraş, 22 sampling stations have been investigated; the data obtained from these stations have been centralised in figure 5. The values presented represent the average for the two years of study (2008 and 2009).

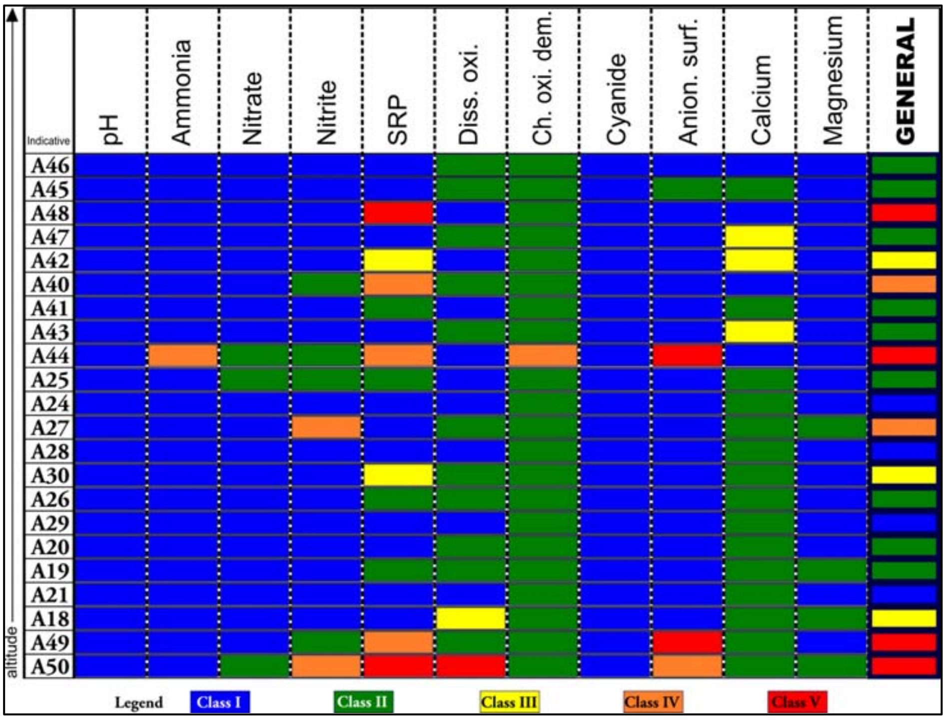


Figure 5: The water classes from the hydrographic basin Bârzava in accordance with the national system of classifying surface waters (O.M.M.G.A. 161/2006)

Indexes for the sampling stations: A18 – Gelugu, A19 – Clocotici amonte, A20 – Nermed, A21 – Caraş, A24 – Oraviţa, A25 – Jitin, A26 – Natra, A27 – Lişava, A28 – Călugăra, A29 – Vicinic, A30 – Cândeni amonte, A40 – Comarnic, A41 – Topliţa, A42 – Răviştea, A43 – Celnicu Mare, A44 – Gârlişte, A45 – Buhui, A46 – Buhui, A47 – Caraş izvoare, A48 – Navăţu Mare, A49 – Cândeni aval, A50 – Clocotici aval

Water classes from the hydrographic basin Nera

Eleven stations have been investigated on the tributaries of the river Nera and two on its main course; the data has been centralised in figure 6. The values presented in the charts represent the average for the two years of study (2008 and 2009).

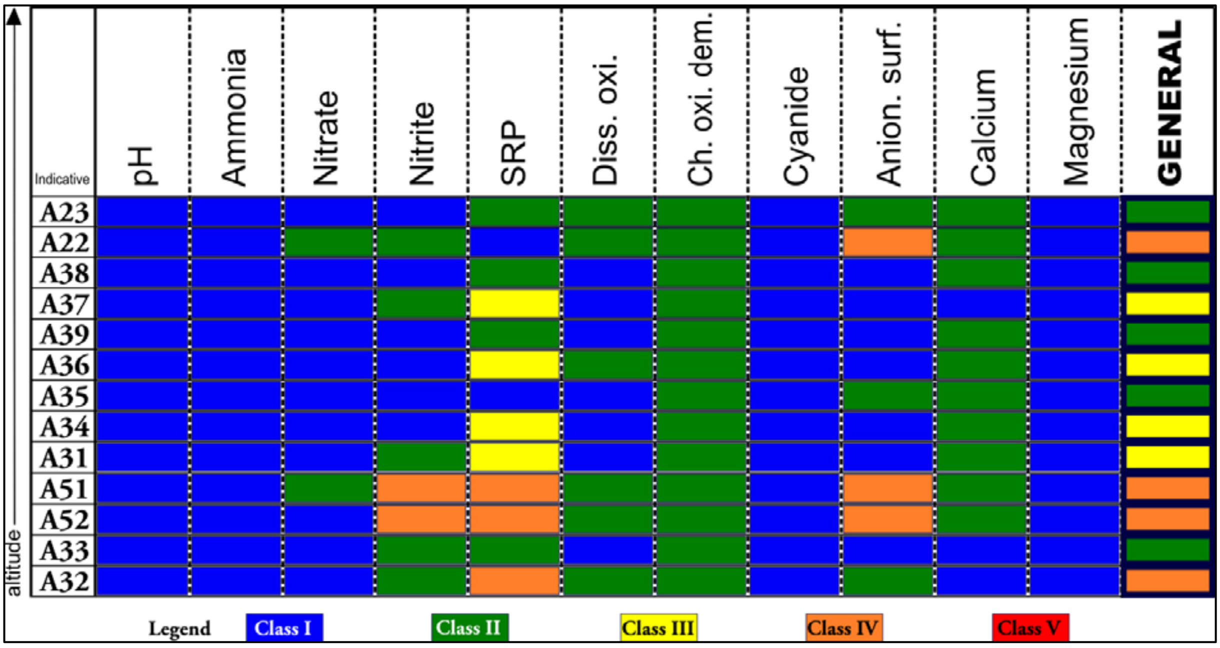


Figure 6: The water classes from the hydrographic basin Bârzava in accordance with the national system of classifying surface waters (O.M.M.G.A. 161/2006)

Indexes for the sampling stations: A22 - Ştaier, A23 – Miniş, A31 – Beiu, ANera, A33 – Bresnic, A34 – Ducin, A35 – Moceriş amonte, A36 – Lăpuşnic amonte, A37 – Poneasca, A38 – Predilcova, A39 – Babii, A51 – Lăpuşnic aval, A52 – Moceriş aval

MATERIALS AND METHODS

Methods used for capturing decapods

In the case of the sweet water decapods, the study implies working with live animals. Thus, we will be referring mainly to capturing methods and not to sampling and preparation methods (Holdich *et al.* 2002, Trouilhe *et al.* 2003, Holdich and Pöckl 2005). The capturing may be active or passive (Dorn *et al.* 2005).

Methods used in the research:

- the direct collection by hand sampling; a number of 115 mature individuals belonging to the species *Austropotamobius torrentium* have been captured as well as 73 mature individuals belonging to the *Astacus astacus* species;
- the capturing that employs traps has been used in this study for the entrapments that led to the identification of the invasive species *Orconectes limosus*.

The collection of the sweet water decapods is forbidden by law. After being captured for investigations all of the individuals have been released back into their natural habitat. The only individuals that have been collected were the 93 specimens found dead in the Cândeni brook, due to some overfall in its bed (Pârvulescu 2009).

Methods used for capturing and collecting aquatic amphipods and isopods

Because we are talking about species whose in field identification is virtually impossible and due to the fact that these groups do not have law protected representatives, their study allows both their capturing as well as the collection of individuals and their transportation into the laboratory. We have applied both qualitative and quantitative methods. For the qualitative method we have used the benthic net, investigating as many microhabitats as possible on the sampling spot. The individuals have been preserved in alcohol 70% or in formaldehyde 4%, inside air-sealed containers. For the quantitative method we have used the Surber benthometer with a surface of 1060 cm^2 disposed with a sieve having an aperture of 250 μm. The individuals thus sampled and washed have been preserved in formaldehyde 4%.

The collection of biometric data

A digital calliper has been used. The measurements apply avoiding juvenilia, the inferior established limit for the total length being 50 mm (Papadopol and Diaconu 1986). The parts of the body that have undergone biometric measurements being those that allow a firm position of the measuring instrument: total length, the length of the cephalothorax, the length of the rostrum, the width of the telson, the maximum thoracic width, the width of the 2nd abdominal segment, the length of the propodite, the length of the dactilopodite, the width of the chela. Measurements have been conducted for 115 individuals belonging to the *Austropotamobius torrentium* species and for 73 individuals belonging to the *Astacus astacus* species.

The measurement of the environmental parameters

Data that can be collected through direct observation, with a minimum energy waste and minimum equipment:

- the approximation of the dimensions of the water body;
- the estimation of the running course;
- the estimation of the type of soil;
- the qualitative estimation of the riparian vegetation (up to the genus level) as well as the degree of shading the major river bed (estimated in percentage);
- the estimation of the habitat neighbouring the water body;
- the description of the capturing microhabitat for each of the important and relevant species or group;
- the estimation of the meteorological conditions characterizing the day of the sampling and the day before the sampling;

Of uttermost importance are the physio-chemical analyses of the water. The working methods and procedures have been the ones that are specific to the type of analysis and to the label of the apparatus employed (table 1).

Table 1: The main technical characteristics for the measured parameters in the evaluation of the surface waters quality from the Anina Mountains

Indicator	Measure units	Interval range	Equipment	Methods
pH	-	1 – 14	HQ 40d	Gel electrolyte
Dissolved oxygen	mg.l-	0.00 – 20.00	HQ 40d	LDO
Water temperature	°C	-10 – +110	HQ 40d	sensor
Conductivity	µS.cm.l-	0,01 – 400	HQ 40d	4 graphite pines
Total hardness Ca + Mg	°dH	1 – 20	DR 2800	Metalftaleine
Chemical oxygen demand	mg.l-	0 – 40	DR 2800	Cromosulfuric acid
Nitrate ($N\text{-}NO_3^-$)	mg.l-	0.1 – 30.0	DR 2800	Cadmium reduction
Nitrite ($N\text{-}NO_2^-$)	mg.l-	0.002 – 0.300	DR 2800	Diazotization
Soluble reactive phosphorus ($P\text{-}PO_4^{3-}$)	mg.l-	0.006 – 0.820	DR 2800	Ascorbic acid
Ammonia ($N\text{-}NH_4^+$)	mg.l-	0.015 – 2	DR 2800	Indophenol blue
Anionic surfactants	mg.l-	0,2 – 2	DR 2800	MBA
Cyanide	mg.l-	0,001 – 0,240	DR 2800	Piridin-pirazoline

The laboratory research

It implies the identification of the species through morphological details, a crucial role being played by the oral pieces and the body's appendage. In parallel we have studied the general shape of the body, the adornment of the urosome and of the epimeral plates. The taking of digital images is highly recommended; these can also later on serve as a visual support for any forthcoming drawings.

Data processing and interpretation

Even if we were only to analyse the statistical data we would come across some very valid conclusions (Shrestha and Kazama 2007, Ouyang *et al.* 2006, Simeonov *et al.* 2003, Payne 1986). The following have been found: the extreme values (minimun and maximum), the arithmetic mean, the standard deviation, as well as various more advanced analysis between clusters of data: the analysis of the dendogrames, the discriminate analysis, the variation (ANOVA). These complex sets of analysis have been conducted with the help of some special software: STATISTICA, PAST and EXCEL.

RESULTS AND DISCUSSION

The list of the aquatic malacostrca species identified in the hydrographic basins of the Anina Mountains

Class Malacostraca Latreille 1802	
Order Decapoda Latreille 1802	*Austropotamobius torrentium* (Schrank 1803)
	Astacus astacus (Linnaeus 1758)

	**Orconectes limosus* (Rafinesque 1817)
Order Amphipoda Latreille 1816	*Gammarus balcanicus* Schäferna 1922
	Gammarus fossarum Koch, în Panzer 1835
	Gammarus roeseli Gervais 1835
Order Isopoda Latreille 1817	*Asellus aquaticus* (Linnaeus 1758)

*invasive species, identified only at the flow of the river Nera into the Danube, with the possibility of being extended

Biometric studies for the decapods

Biometric studies have been conducted on individuals belonging to the decapoda order. The ratio between the sexes of the captured individuals (sex-ratio) seems to favour females for the *Austropotamobius torrentium* species, having been found a male for 1,017 females, while the ratio for the *Astacus astacus* species favours the male individuals, having been identified a male for 0,871 females.

The discriminate analysis reveals the most obvious differences between the two sexes. These differences are represented by the dimension of the chelas, which is similar with the results obtained in 1987 by Papadopol and Diaconu, on a sample of 27 individuals.

The maximum dimension of the individuals belonging to the *Austropotamobius torrentium* species concerning the total length (TL) varies between similar limits to both males and females, the largest male having a total body length of 93,34 mm. In what the width of the 2nd abdominal segment is concerned (W2A) the medium value is greater in the case of the female individuals. The maximum dimension of the total length (TL) for the *Astacus astacus* species is bigger for the male individuals, the largest male individual 127, 34 mm, while the largest female individual measured reached the value of 108, 80 mm. All of the medium values measured are larger for the male individuals as compared to female individuals.

In order to compare the populations of the three hydrographic basins of the Anina Mountains, we have resorted to a variation analysis test (ANOVA). Since the distribution of the investigated species has not been uniform in the three hydrographic basins that we have analysed, we have made use only of the data collected from the captured individuals in the stations that have had a representative number. For the *A. torrentium* species the strongest discriminate character is represented by the following value groups: propodite length – dactilopodite length (PL-DL), propodite length – chela width (PL-CW) and dactilopodite length – chela width (DL-CW). For the *A. astacus* species the strongest discriminant character is represented by the same value groups like in the case of the prior species, as well as the pair of parameters total length – cephalothorax length (TL-CtL). According to the variance analysis the results for the female groups of the *A. torrentium* species found in the three investigated basins do not satisfy the confidence limit ($p \leq 0,05$) for none of the three estimated ratios. However, a certain resemblance may be noted between the populations of the Nera and the Bârzava basins. The male individuals show statistically significant differences ($p \leq 0, 05$) concerning the PL/CW ratio (figure 7).

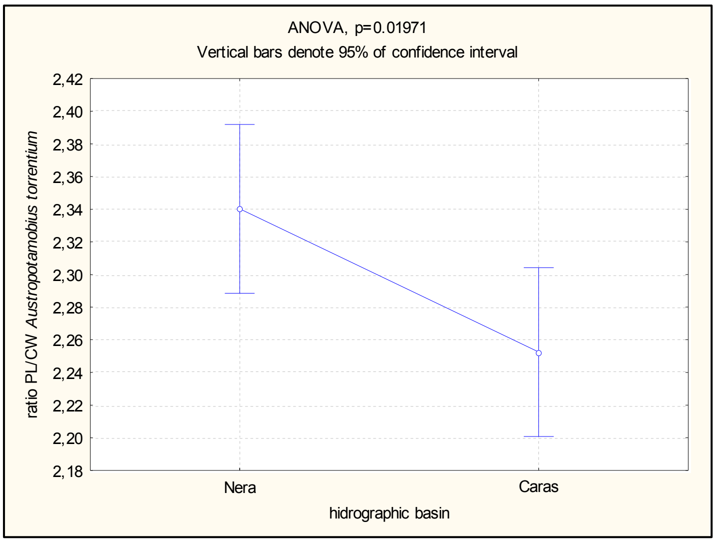


Figure 7: The ANOVA test for the male individuals of *A. torrentium* from the hydrographic basins Nera and Caraş, for the PL/CW ratio

In what the *A. astacus* species is concerned, we may notice that the female groups from the two investigated basins are significantly different in their ratios between the length of the propodite and the width of the chela, as well as between the length of the dactilopodite and the width of the chela (figure 8 and 9).

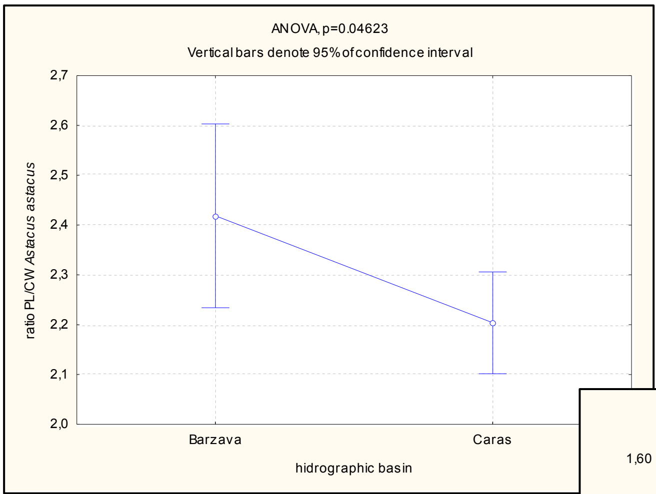


Figure 8: The ANOVA test for the female individuals of *A. astacus* from the hydrographic basins Nera and Caraş, for the PL/CW ratio

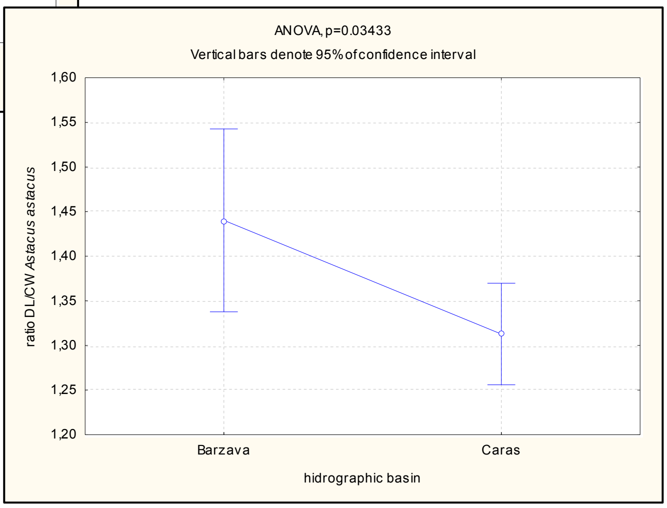
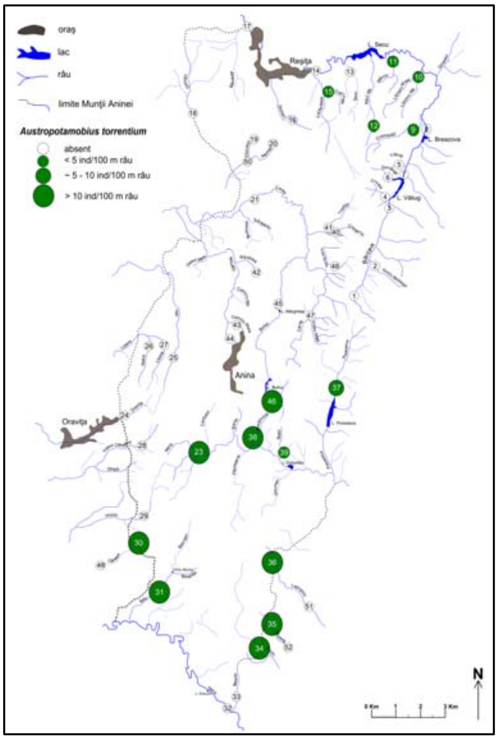


Figure 9: The ANOVA test for the female individuals of *A. astacus* from the hydrographic basins Nera and Caraş, for the DL/CW ratio

The distribution of the aquatic malacostraca species in the Anina Mountains

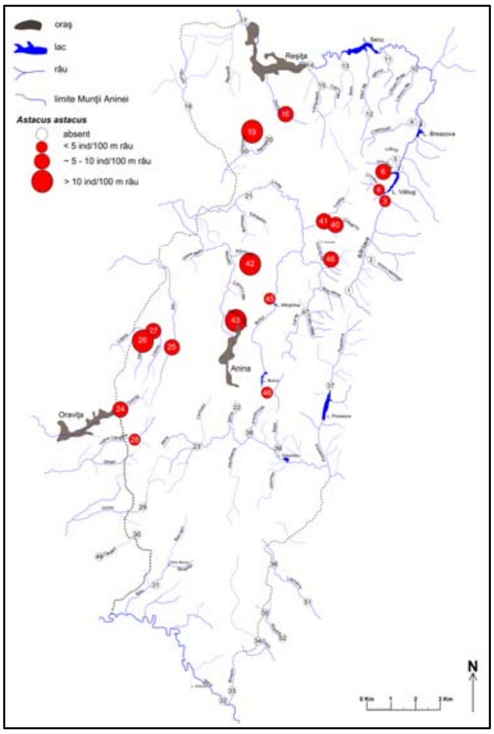
Austropotamobius torrentium (Schrank 1803)

It has been identified in all the three hydrographic basins, being best represented at the level of the hydrographic basin of the river Nera. In the basin of the river Bârzava, the species has been found only on the river's tributaries that are on the upstream of the reservoirs Văliug and Breazova. In all of these stations, the relative abundance of these species has been under the value of 5 individuals for 100 metres of investigated river. In the hydrographic basin of the river Caraş this decapod is only poorly represented, its place being taken by the *Astacus astacus* species. However, we have met the species in two locations placed in the superior area. Being the only decapod species within the basin of the river Nera, it is very well represented. Populations with a high numeric abundance of over 10 individuals/100 m of investigate driver have been found in numerous brooks.



Astacus astacus (Linnaeus 1758)

The species has been identified in two of the three hydrographic basins, namely in: Bârzava and Caraş, being best represented in the basin of the river Caraş. In the hydrographic basin of the river Bârzava, the *A. astacus* species has been captured only near the reservoir Văliug and in the tributary Doman. For the hydrographical basin of the river Caraş, it has been noticed that this species is very well represented, populations with a high numeric abundance being registered in numerous brooks. At the station Buhui springs the species has been found in cohabitation with the decapod *Austropotamobius torrentium*, this being the dominant species.



Orconectes limosus (Rafinesque 1817)

The investigations we have conducted near the Anina Mountains have proved the presence of the invasive species *Orconectes limosus* located in the area where the river flows into the Danube, namely in Balta Nera. Having extended afterwards the investigations through the observation of the Danube upstream of Baziaş, the invasive species has been found all the way to the Berzasca area, around 1018 kilometre as compared to Danube's flow into the Black Sea (figure 10).

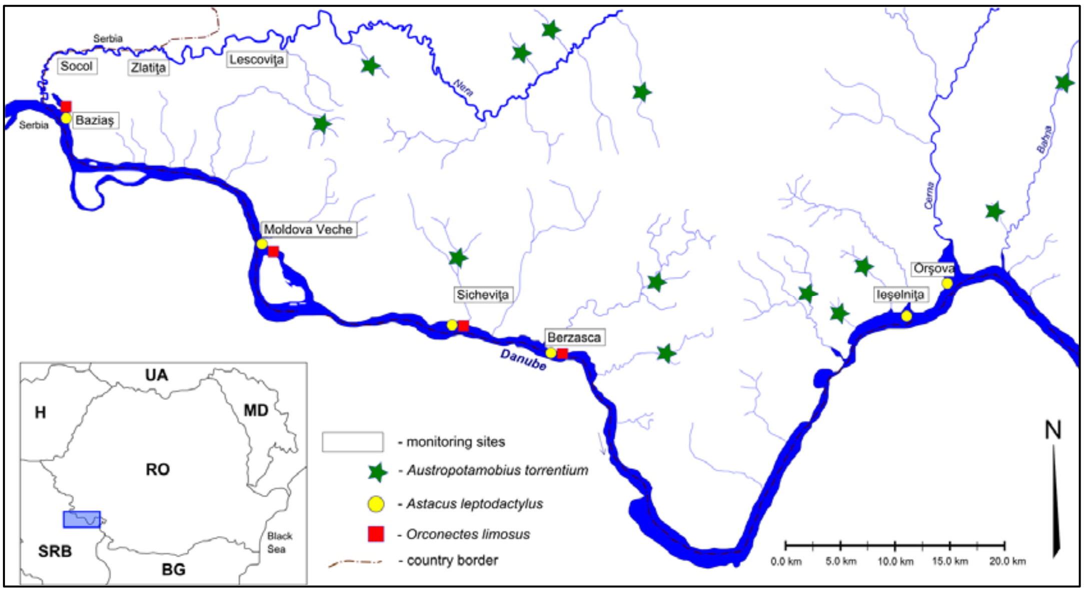
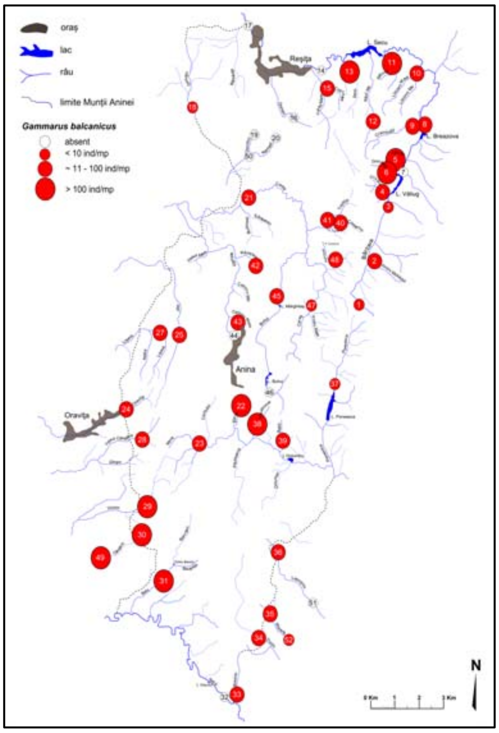


Figure 10: The observation and presence points of the invasive species *Orconectes limosus* (Pârvulescu *et al.* 2009)

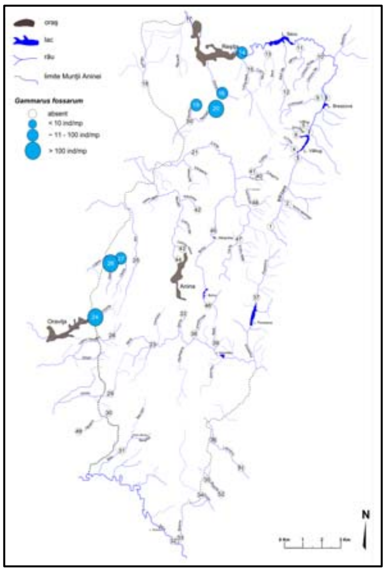
Gammarus balcanicus Schäferna 1922

As revealed by the investigations we have conducted in the aquatic lotic habitats of the Anina Mountains, it can be stated that this is the commonest species of all of the aquatic malacostraca found in this region, the frequency with which it has been encountered within the stations from the Anina Mountains reaching the value of 75% from the total of the 52 stations. In the hydrographic basin of the river Bârzava, the *Gammarus balcanicus* species have been found in numerous stations placed on the main course of the river in the superior area, as well as on its tributaries. For the hydrographic basin of the river Caraş, the distribution of this species shows its presence both within the stations placed on the main course of the river and on the river's tributaries. With the exception of the main course of the river Nera, the species has been found in all of the other stations.



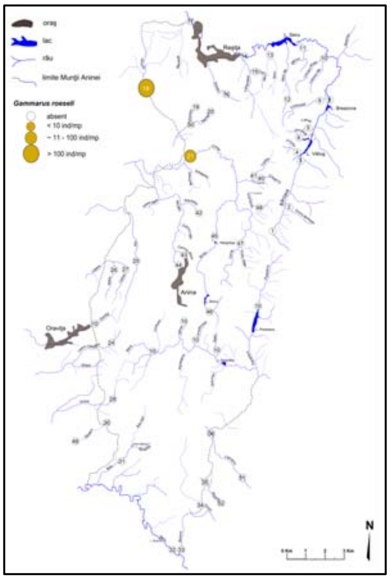
Gammarus fossarum Koch, in Panzer 1835

Our observations show the presence of this amphipod species in the north-western part of the Anina Mountains, within the hydrographic basins of the rivers Bârzava and Caraş. For the hydrographic basin of the river Bârzava, the species has been identified only in the sampling collected from two of the stations: Bârzava on the upstream of the cities Reşiţa and Doman. It is better represented in the hydrographic basin of the river Caraş. In two of the investigated locations (Oraviţa and Lişava) this species cohabitates with another amphipod species, *Gammarus balcanicus*.



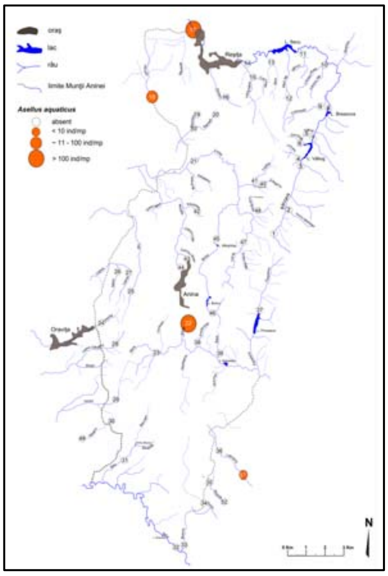
Gammarus roseli Gervais 1835

Our observations prove that this species lives in only two brooks situated in the hydrographic basin of the river Caraş, in brook Gelugu and in the river Caraş upstream the gorge (figure 78). In both stations the species cohabitates with *Gammarus balcanicus*; however, the latter is much more poorly represented.



Asellus aquaticus (Linnaeus 1758)

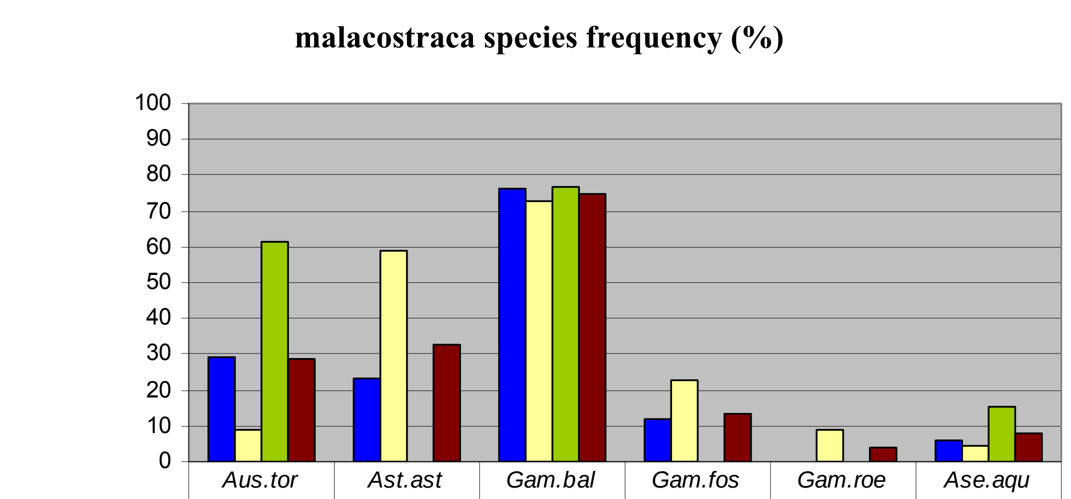
The species has been identified in all of the three hydrographic basins, but with a very low frequency (figure 78). For the hydrographic basin of the river Bârzava, we signal the presence of this species only at the station placed on the upstream of the city Reşiţa, location in which the density of this species surpasses 100 individuals per square metre. The species has been found only at one of the stations placed in the hydrographic basin of the river Caraş, namely the station place don the brook Gelugu. In the hydrographic basin of the river Nera the species has been discovered in two of the 13 stations.



The qualitative and quantitative ecological characteristics

Frequency

Both of the decapod species identified have been encountered with a frequency of fewer than 35% from the total of the 52 investigated stations from the Anina Mountains. The *Austropotamobius torrentium* species (present in all of the three basins) has been found to have the highest frequency in the hydrographic basin of the river Nera. The *Astacus astacus* species has been found with a higher frequency in the basin of the river Caraş, but it was proved to be missing from the waters of the basin Nera. In what the amphipods are concerned, the most frequently found species within the stations of the Anina Mountains is the *Gammarus balcanicus*. The other two species, *Gammarus fossarum* and *Gammarus roeseli* have been found at the level of the entire mountain with a lower frequency (figure 11).



	Aus.tor	Ast.ast	Gam.bal	Gam.fos	Gam.roe	Ase.aqu
Barzava	29.4	23.5	76.4	11.7	0	5.8
Caras	9.1	59.1	72.7	22.7	9.1	4.5
Nera	61.5	0	76.9	0	0	15.3
Muntii Aninei	28.8	32.6	75	13.4	3.8	7.7

Figure 11: The frequency of the malacostraca species within the hydrographic basins and entire mountain
Abbreviations: *Aus.tor = Austropotamobius torrentium; Ast.ast = Astacus astacus*; *Gam.bal = Gammarus balcanicus; Gam.fos = G. fossarum; Gam.roe = G. roeseli; Ase.aqu = Asellus aquaticus*

The density of the amphipod and isopod species

Gammarus balcanicus is the best represented species in this area. In the hydrographic basin of the river Bârzava, populations with a density of over 100 individuals per square metre have been found on the tributaries: Văliug, Grindeşti, Stârnic and Secu. For the hydrographic basin of the river Caraş, populations with over 100 individuals per square metre have been discovered in the southern area of this basin, in the tributaries Cândeni and Vicinic. In the hydrographic basin of the river Nera the species has been found in populations with a high density in the following stations: Ştaier, Beiu and Predilcova. *Gammarus fossarum* is an amphipod species that has been found in the western and north-western part of the Anina Mountains, in the hydrographic basin of the rivers

Bârzava and Caraș. In the hydrographic basin of the river, the density of the individuals per square metre has been lower than 100 individuals for both of the stations where it has been identified. Very well represented populations have been identified in the hydrographic basin of the river Caraș, in the tributaries Nermed, Oravița and Natra. In the hydrographic basin of the river Bârzava, at the only station placed there the density of the *Asellus aquaticus* species has been calculated at 125,12 ind/m^2. The species has been discovered in one of the stations found in the hydrographic basin of the river Caraș, namely at the Gelugu station, but with a lower density. In the hydrographic basin of the river Nera, the species has been found in two stations. At the station that is place don the brook Ștaier high values for the population's density have been registered, a density that has been estimated at 146, 93 ind/m^2.

<u>Numeric abundance</u>

The decapod *Austropotamobius torrentium* in the basin of the river Bârzava has generally been found in a low numeric abundance, the best populated station being the Stârnic station, registering the figure of 1, 94 individuals/100 m of investigated river. The poorest numeric abundance has been calculated for the Liscov station, with 0, 5 individuals/100 m of investigated river. In the two stations from the basin of the river Caraș, the numeric abundance has been very high for this species, at the Cândeni station being captured per average 32, 08, and at the station Buhui springs 12, 5 individuals/100 m of investigated river. The best represented populations for this species have been found in the hydrographic basin of the river Nera, at the Lăpușnic station, placed upstream the village with 63,33 individuals/100 m of investigated river. The decapod *Astacus astacus* registers well represented populations in the basin of the river Bârzava, in the stations Crivaia and Grindești, the numeric abundance being estimated at 11 individuals/100 m of investigated river. In the basin of the river Caraș, populations with a numeric abundance of over 20 individuals/100 m of investigated river have been discovered in the brook Clocotici, upstream the villages Clocotici, Răviștea and Natra. A low numeric abundance has been calculated for the brooks Valea Călugăra and Buhui springs, with approximately 1 individual/100 m of investigated river.

<u>The numeric dominance of large malacostraca</u>

As far as the decapods are concerned, in the montane and submontane region of the Anina Mountains populations have been represented by one species only on a water course. The two species coexist in the same brook only in the station Buhui springs, upstream the Buhui Lake. The dominant species is the *Austropotamobius torrentium* registering 94,69% of the total amount of captured species at this station.

The numeric dominance of small malacostraca

Small malacostraca (amphipods, isopods) have been found in cohabitation in 5 of the stations found in all of the three investigated basins. The station with the highest diversity of species was the station Gelugu, from the basin of the river Caraş, where three species have been identified, two amphipods: *Gammarus balcanicus* and *Gammarus roeseli*, and the isopod *Asellus aquaticus* (figure 12).

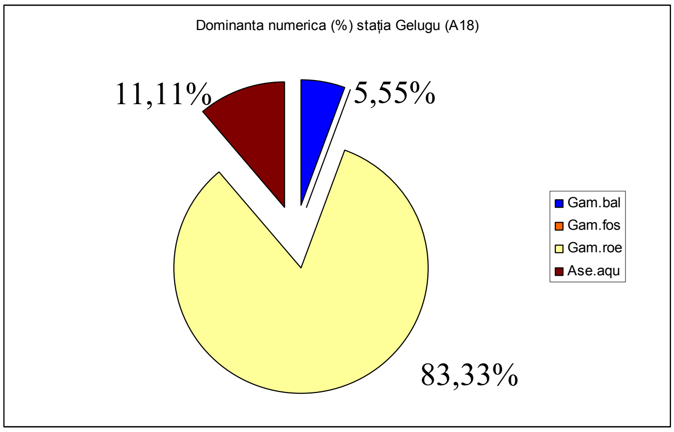
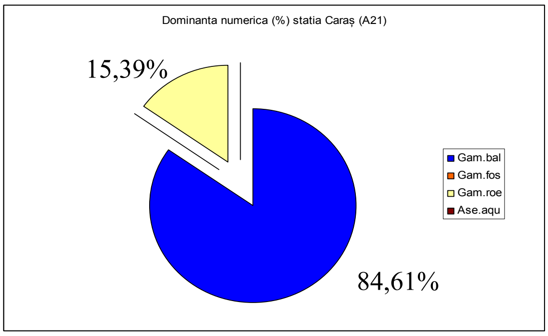
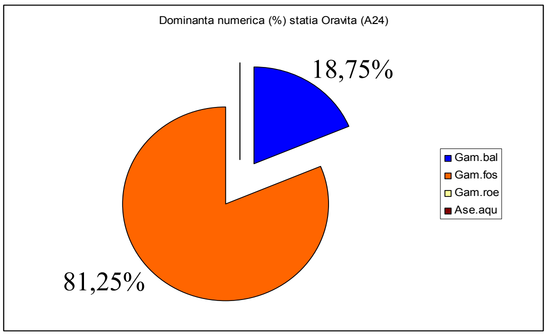


Figure 12: The numeric dominance of small malacostraca, collected from the Gelugu station (for abbreviations see figure 11)

Another station where small malacostraca species cohabitate is the station on the river Caraş, on the upstream of the Cheile Caraşului, here being found the amphipods *Gammarus balcanicus* and *Gammarus roeseli* (figure 13). The species *Gammarus balcanicus* and *Gammarus fossarum* (figure 13) have been found in cohabitation in two of the stations placed in the Caraş basin.





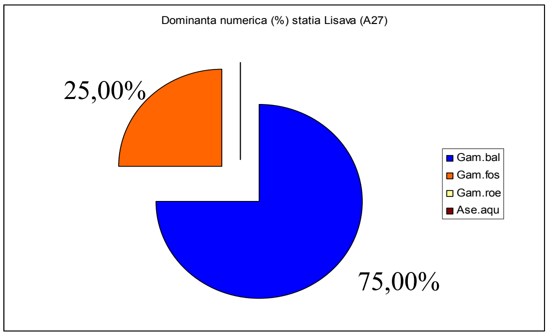


Figure 13: The numeric dominance of small malacostraca, collected from the Caraş station - left, Oravița station - right, Lișava station – center (for abbreviations see figure 11)

In the basin of the river Nera, only one location has been discovered to have small malacostraca species that live in cohabitation, namely the Ştaier station, with the species *Gammarus balcanicus* and *Asellus aquaticus* (figure 14).

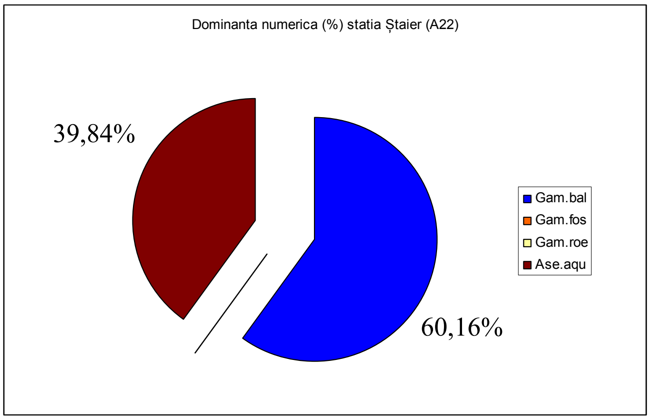


Figure 14: The numeric dominance of small malacostraca, collected from the Ştaier station (for abbreviations see figure 11)

Factors that influence the distribution of the aquatic malacostraca in the Anina Mountains

The distribution of the species is influenced by certain environmental factors (Hudina *et al.* 2008, Naura and Robinosn 1998). In this way, the values of the physical and chemical parameters measured in the field have been the subject of analyses and statistic calculations that were conducted separately for each of the species pointing out the preference for the habitats in which the analysed species has been found. In order to achieve this we shall make a distinction in our discussion between the physical factors, the chemical ones and the anthropic impact in the investigated area.

Physical factors

<u>The altitude</u> for the species belonging to the decapod order plays an important part only in the distribution of the *A. astacus* species, the area in which this species has been discovered ranging from 360 and 480 m; this is a significantly different span as compared to the span registered in the stations where the species has not been found (figure 15 - left). In what the *A. torrentium* species is concerned we have noticed that altitude is not a restricting factor for its distribution in the waters of the Anina Mountains (figure 16 – right).

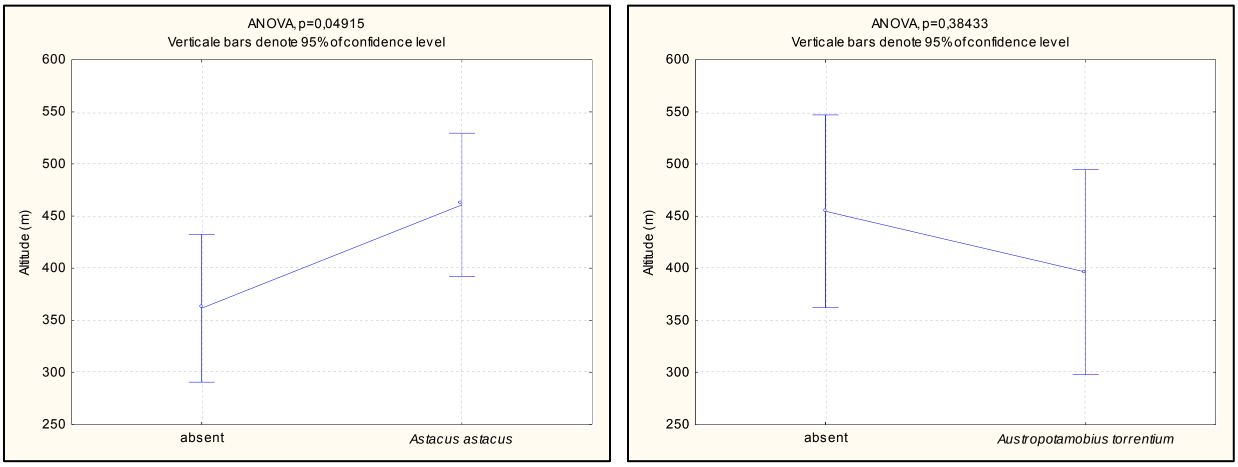


Figure 16: The ANOVA tests for altitude (m), *Astacus astacus* - left; *Austropotamobius torrentium* – right

The statistic analysis of the data collected on altitude for the amphipod species shows that very closet o the confidence limit are the results for the species *G. balcanicus* and *G. fossarum*. The medium altitude where the *G. balcanicus* has been collected is around 425 m. For *G. fossarum* the average altitude is set around the value of 300 m, and for the *G. roeseli* species, due to the small number of stations (two) in which the species has been discovered we consider the result of the statistic analysis as irrelevant; the same situation is also registered for the isopod *Asellus aquaticus*.

The water flow velocity for the *Astacus astacus* shows a greater preference for the habitats with a high flow velocity (figure 17). The same tendency to populate habitats with well oxygenised water can also be noticed for the *Austropotamobius torrentium* species, (figure 17). The preferences for the *Gammarus balcanicus* species are to a lesser degree related to a certain flow type (figure 17). For the species *Gammarus fossarum* and *G. roeseli*, the preferences are linked to waters with a slow flow; the same is true about the isopod species *Asellus aquaticus* (figure 17).

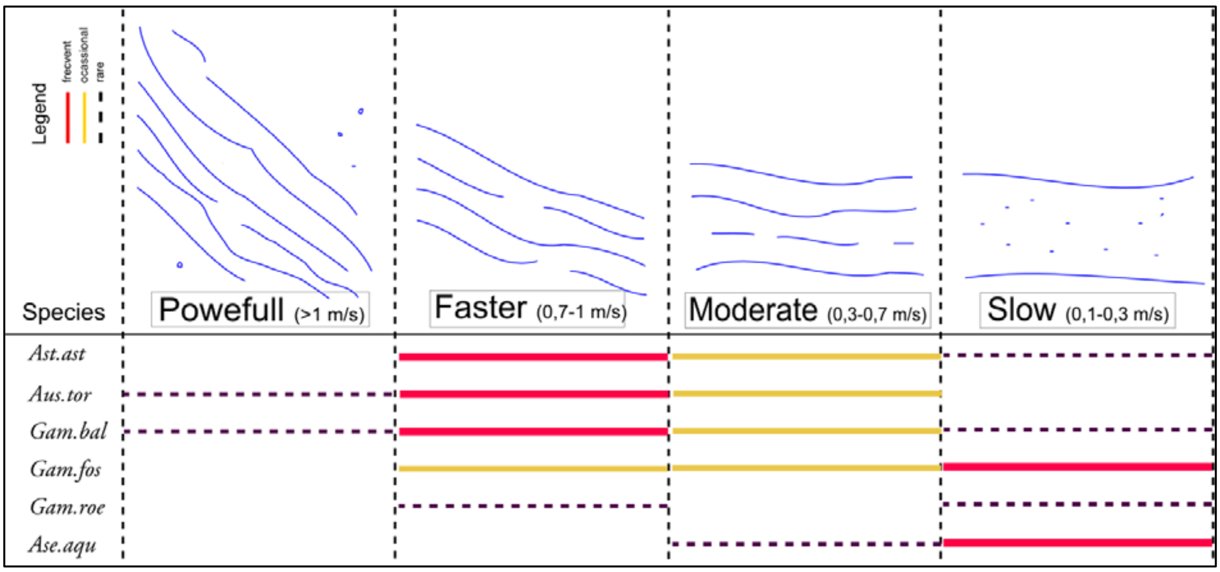


Figure 17: The distribution of the malacostraca species in relation to the water flow velocity. The marks represent the frequency: red – over 50%, yellow – 10-50 %, dotted line – under 10%. (for abbreviations see figure 11)

The width of the minor stream bed. Analysing the data of the average width of the minor stream bed for the investigated waters we notice that the majority of the *A. astacus* individuals have been captured in areas where the river bed measured between 1 and 4,5 m, while for the *A. torrentium* the majority of the individuals have been found in areas with a width between 1 and 2 m; the average of all of the stations placed in the Anina Mountains being around 3 m. These results can be explained by the fact that the *A. torrentium* prefers habitats that are closer to small brooks which are less exposed to spring floods (Vlach *et al.* 2009).

The substratum of the minor stream bed. As can be seen in figure 18 the majority malacostraca species from the Anina Mountains have been found on a substratum made up mainly of pebbles and grovel; this type of substratum allows the finding of refugees for these invertebrate.

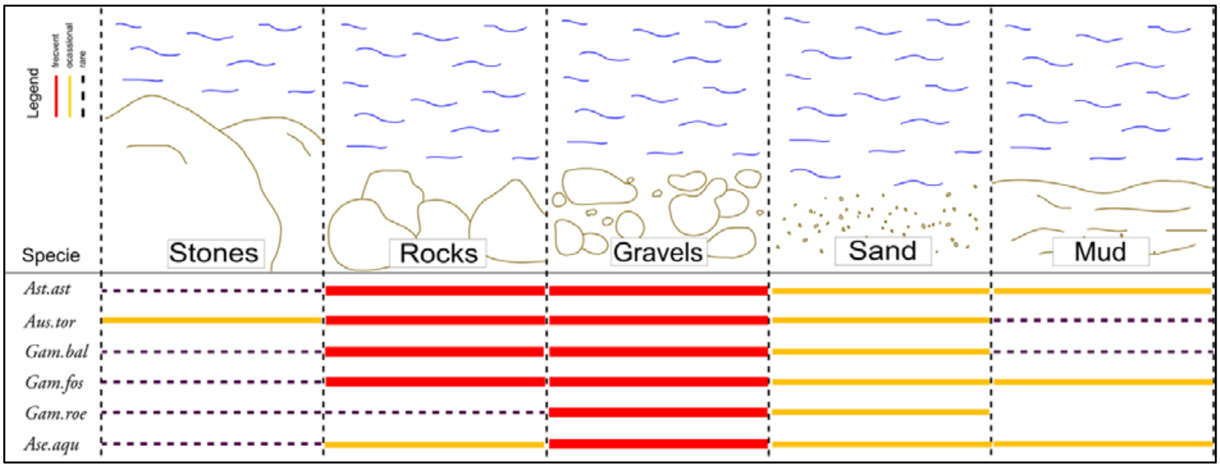


Figure 18: The distribution of the malacostraca species in relation to the granulation of the substratum (for abbreviations see figure 17)

The shadowing level of the major stream bed. Analysing the data concerning the covering of the stream bed with perennial vegetation, we can observe that the malacostraca species prove to have a tendency towards the habitats with a high degree of shadowing, in most of the cases being found at a covering larger than 70% (figure 19).

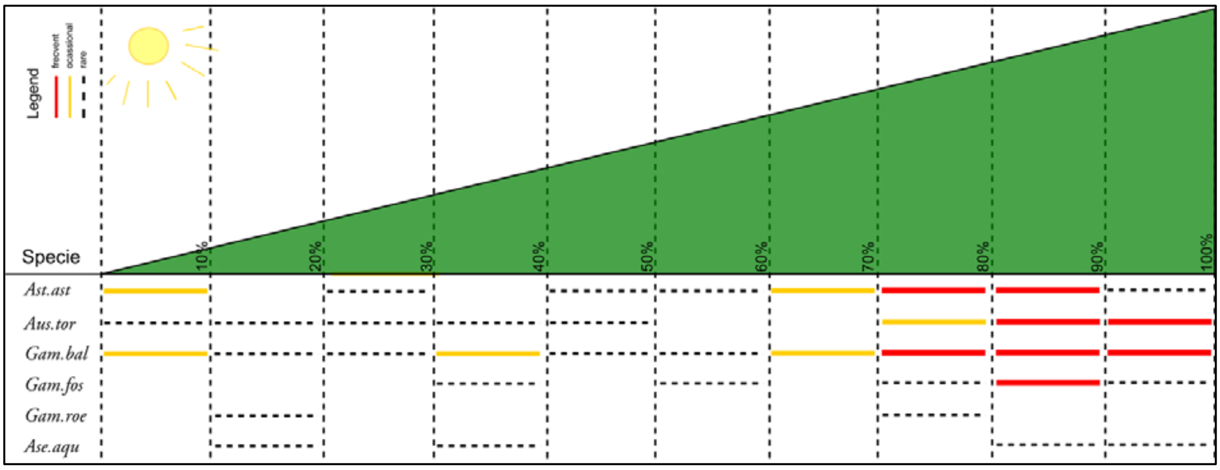


Figure 19: The distribution of the malacostraca species in relation to the shadowing level of the stream bed (for abbreviations see figure 17)

The habitat type found around the major stream bed. As far as the distribution of the species in accordance to the habitat around the sampling stations is concerned, we may say that the level of the mixed and deciduous forests is preferred by all of the malacostraca species identified in the Anina Mountains (figure 20).

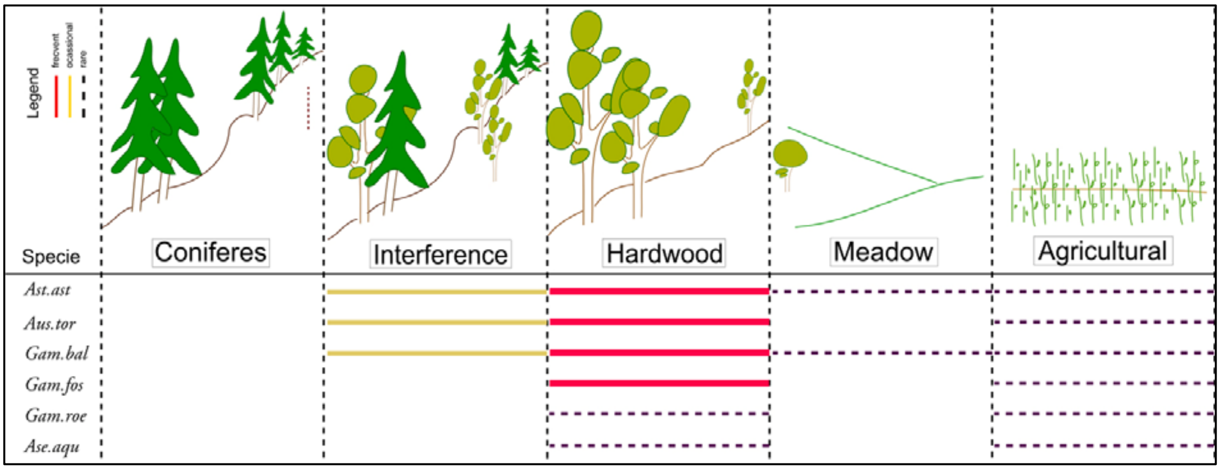


Figure 20: The distribution of the malacostraca species in relation to the habitat type found around the stream bed (for abbreviations see figure 17)

Chemical factors

In our trial to establish which of the water's chemical parameters analysed at the 52 investigated stations, have a certain effect on the distribution of the malacostraca species, we shall discuss next the qualitative data for each of the species through the test of the variance analysis (ANOVA) comparing the data for each of the parameters from the station in which the species has been found with the data from the station in which the species has not been (Muxik *et al.* 2007).

The relation between the chemical composition of the water and the decapod species

For the *Astacus astacus* the ANOVA test does not have significant results for none of the measured parameters; however, close to the confidence limit are placed the values for nitrite, for the chemical oxygen demand and for the anionic surfactants (figure 21-23).

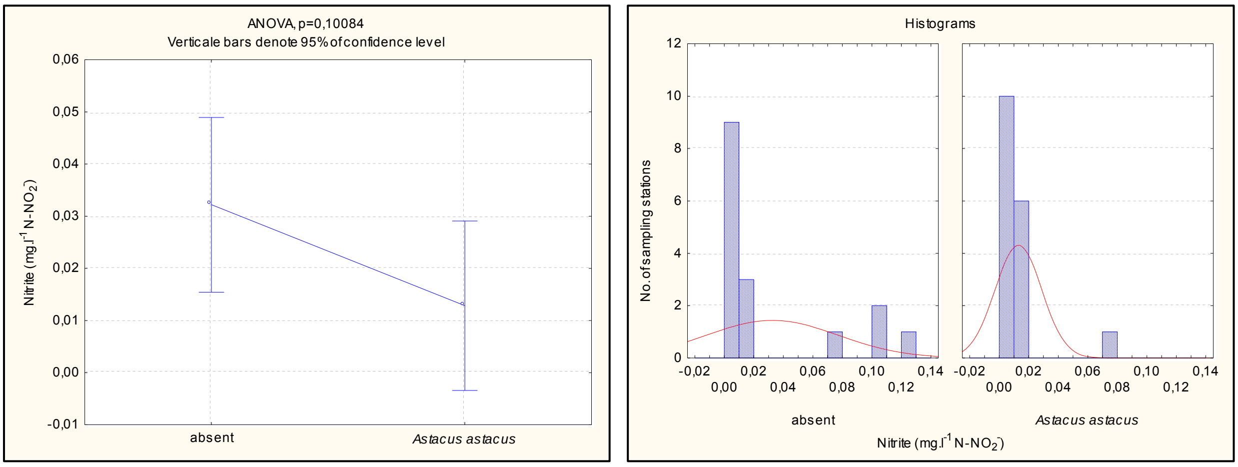


Figure 21: The ANOVA test (left) and the histograms (right) for the absence/presence of the *A. astacus* species, for nitrite

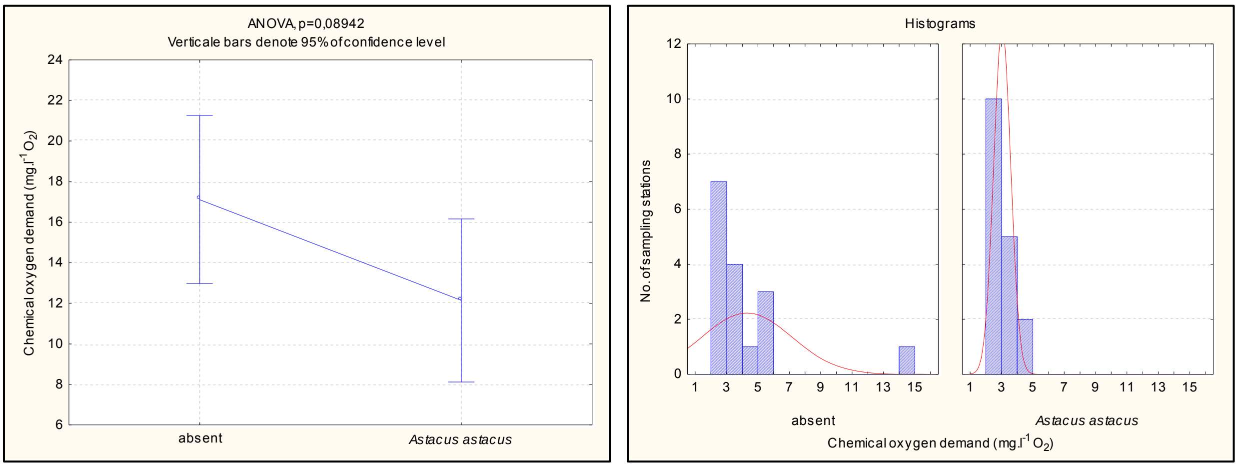


Figure 22: The ANOVA test (left) and the histograms (right) for the absence/presence of the *A. astacus* species, for chemical oxygen demand

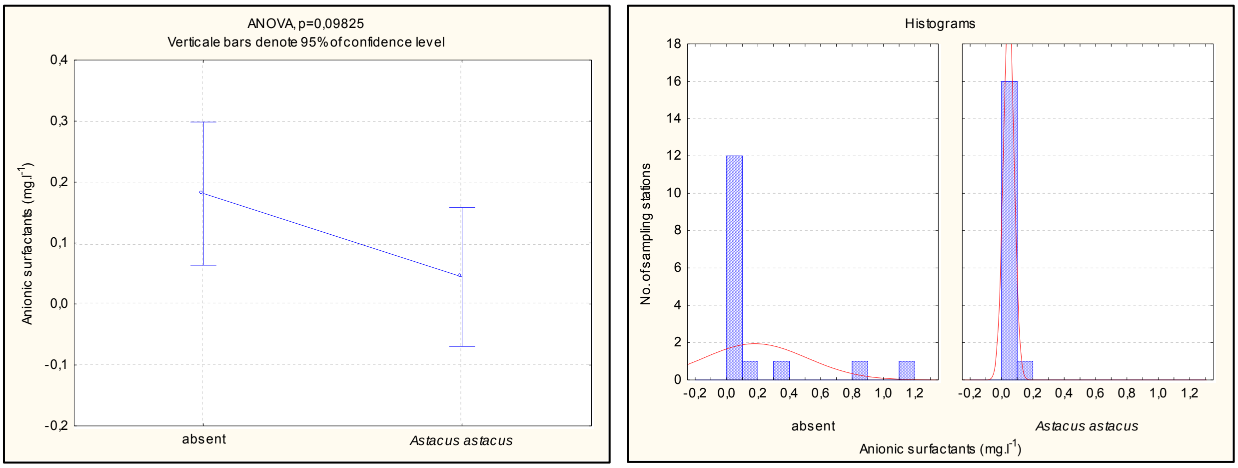


Figure 23: The ANOVA test (left) and the histograms (right) for the absence/presence of the *A. astacus* species, for anionic surfactants

In the case of the *Austropotamobius torrentium* species, the ANOVA test reveals significant differences for the set of values of the pH and of the nitrate (figure 24, 25).

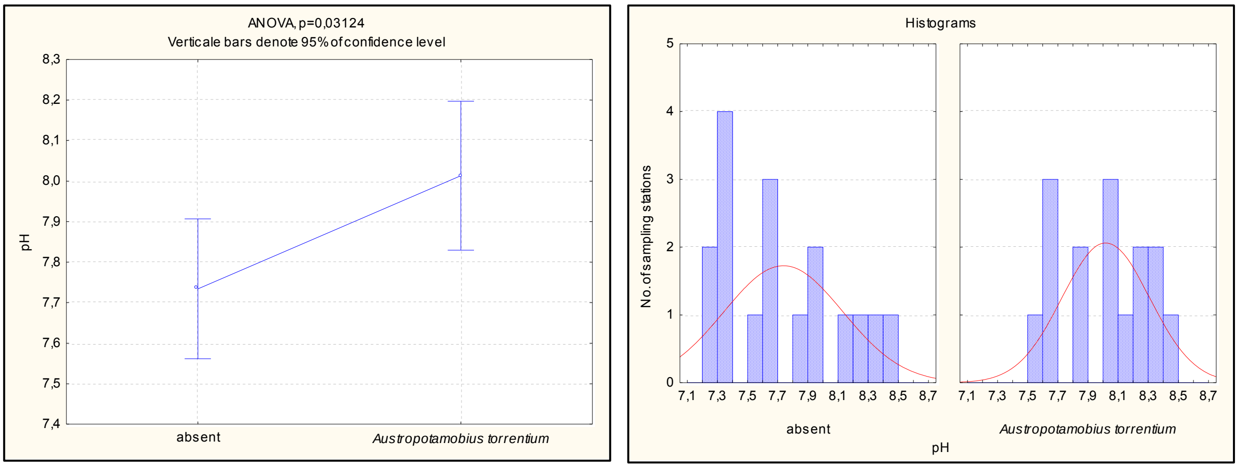


Figure 24: The ANOVA test (left) and the histograms (right) for the absence/presence of the *A. torrentium* species, for pH

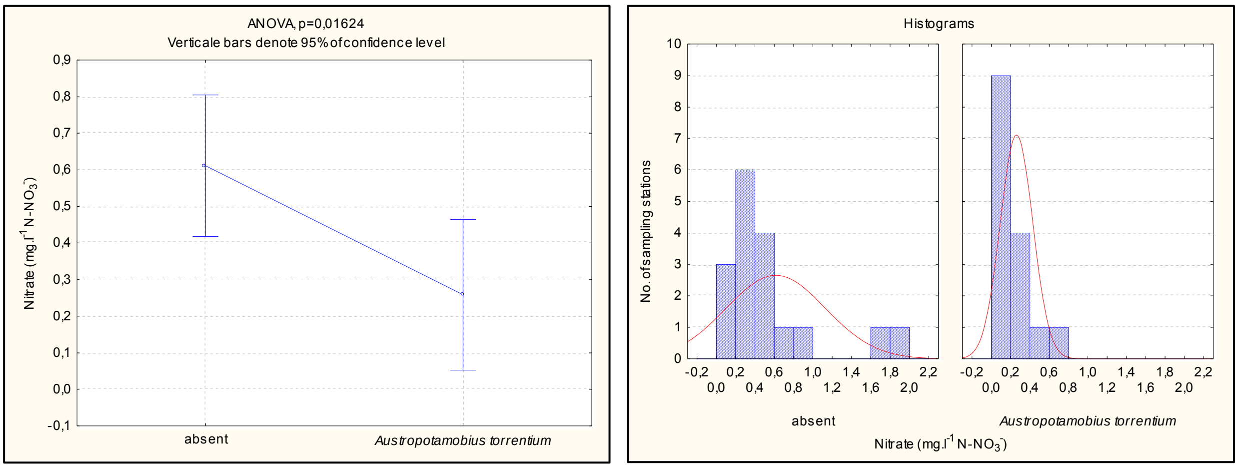


Figure 25: The ANOVA test (left) and the histograms (right) for the absence/presence of the *A. torrentium* species, for nitrate

Values closet o the confidence limit has also been registered for nitrite and for dissolved oxygen (figure 26, 27).

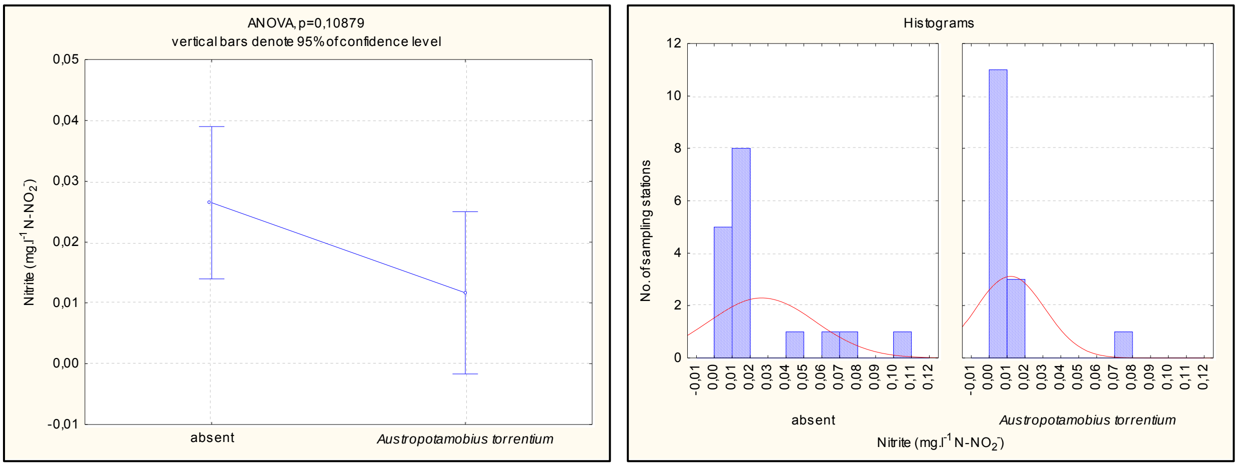


Figure 26: The ANOVA test (left) and the histograms (right) for the absence/presence of the *A. torrentium* species, for nitrite

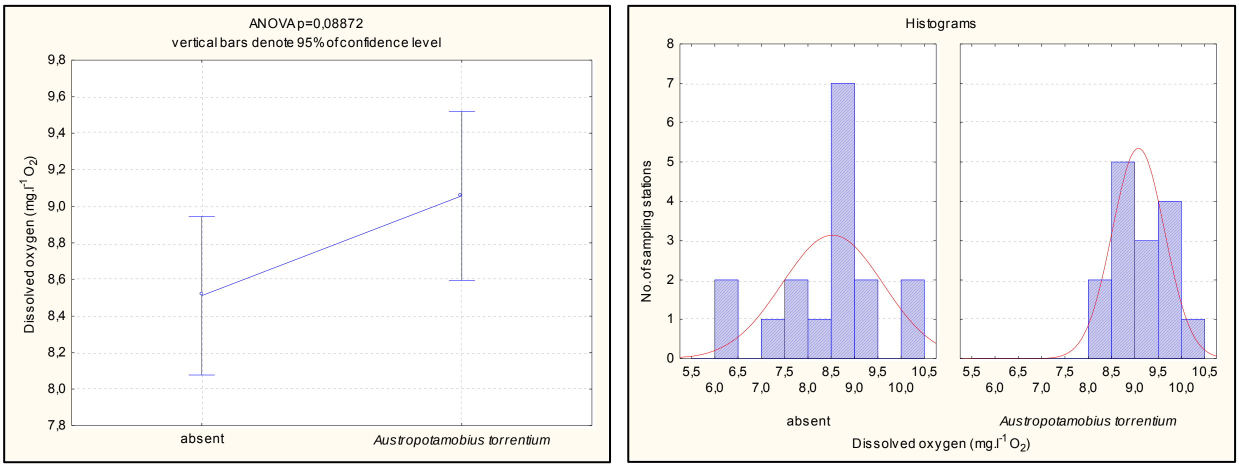


Figure 27: The ANOVA test (left) and the histograms (right) for the absence/presence of the *A. torrentium* species, for dissolved oxygen

A comparison between the two species according to the data measured in the locations where they live shows a difference placed very close to the relevance limit only for nitrate, the *A. torrentium* species being less tolerant (figure 28).

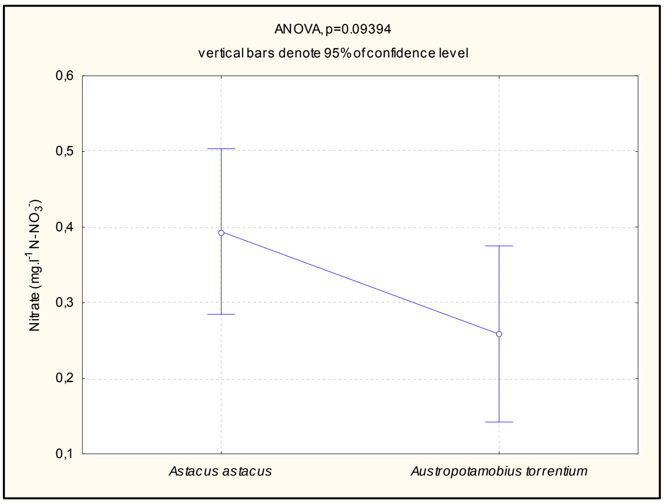


Figure 28: The ANOVA test for nitrate, between the stations with *A. astacus* and the ones with *A. torrentium*

In spite of the results obtained, along with the results of other authors (Laurent 1985, Foster and Turner 1993, Troschel 1997, Broquet *et al.* 2002, Trouilhe *et al.* 2002), the cause for which the decapod species lack from some of the stations where the water quality is very good cannot be proved. A possible explanation could be the poor water quality of the main courses, this situation making it impossible to migrate to new territories.

<u>The relation between the chemical composition of the water and the amphipode species</u>

For the *Gammarus balcanicus* the test shows significant results for nitrite, soluble reactive phosphorus, dissolved oxygen, chemical oxygen demand, magnesium (figure 29-33) as well as results placed close to the confidence limit.

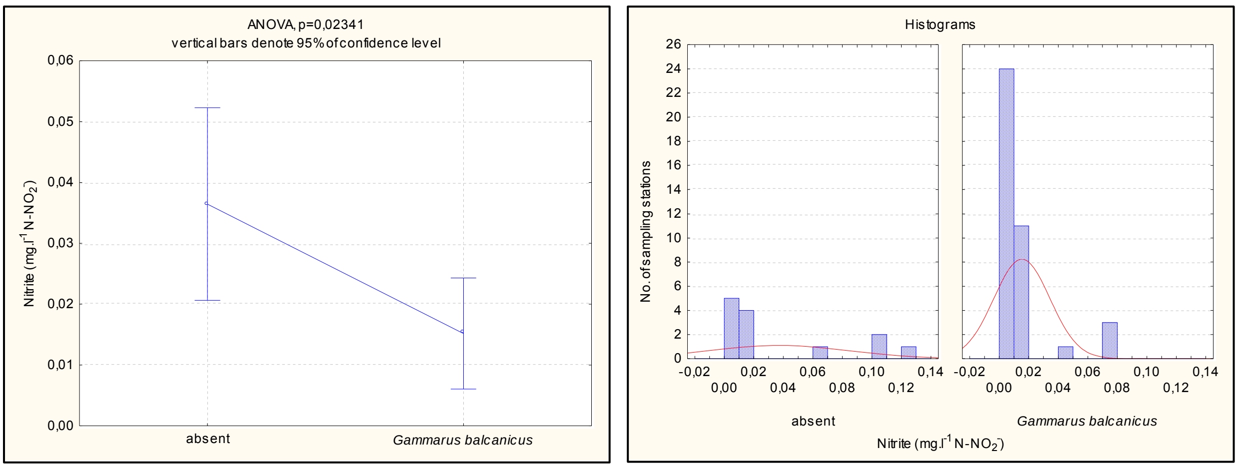


Figure 29: The ANOVA test (left) and the histograms (right) for the absence/presence of the *G. balcanicus* species, for nitrite

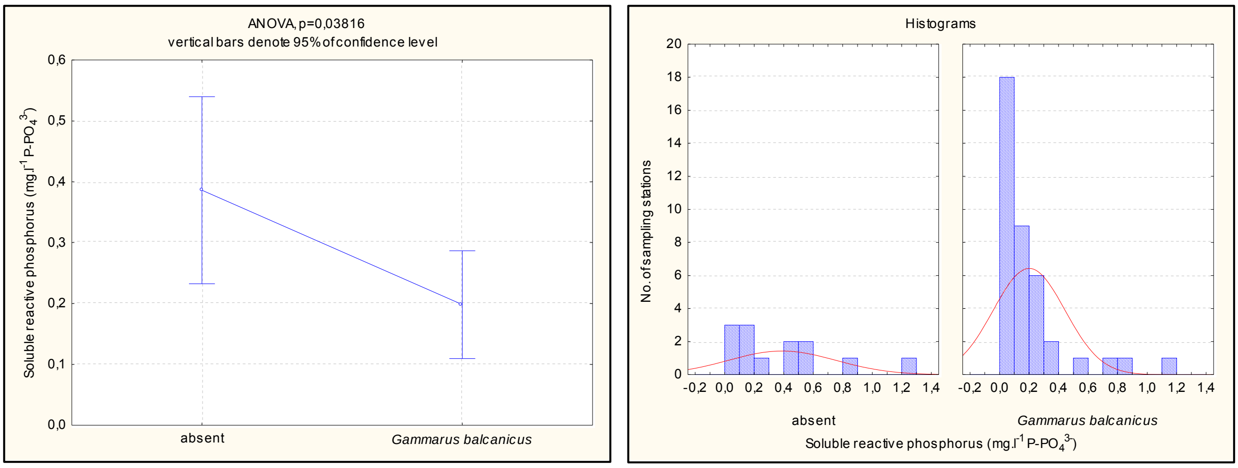


Figure 30: The ANOVA test (left) and the histograms (right) for the absence/presence of the *G. balcanicus* species, for soluble reactive phosphorus

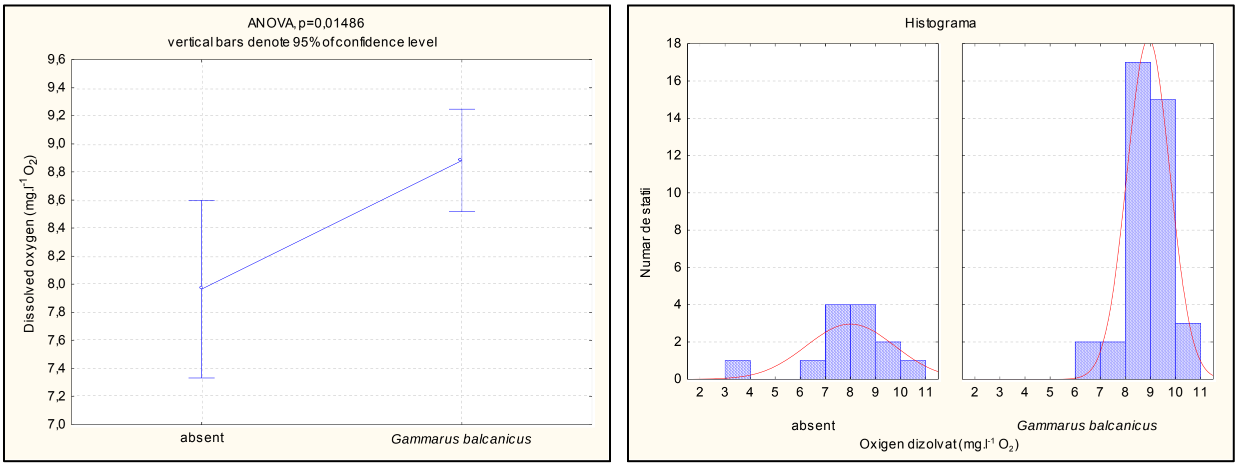


Figure 31: The ANOVA test (left) and the histograms (right) for the absence/presence of the *G. balcanicus* species, for dissolved oxygen

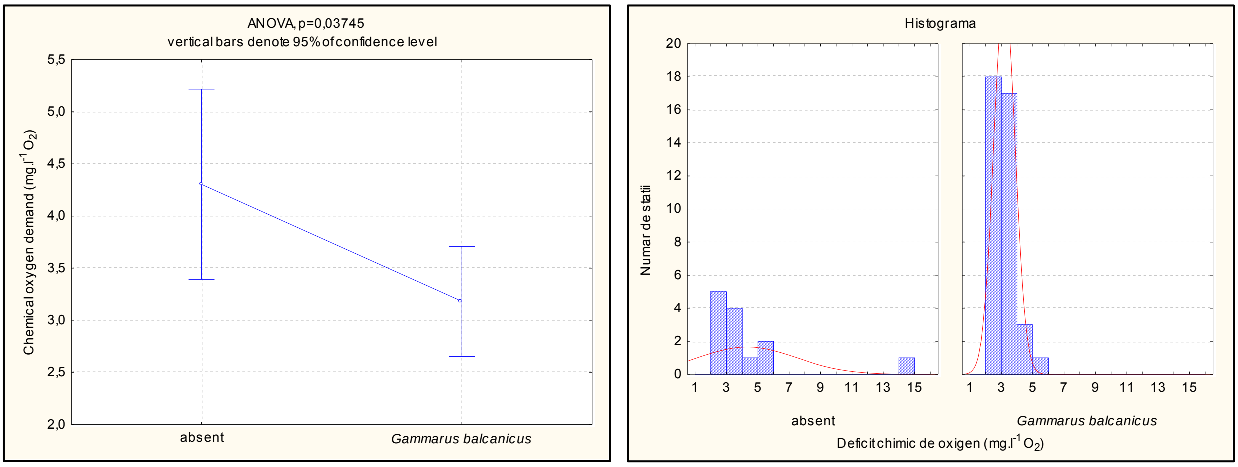


Figure 32: The ANOVA test (left) and the histograms (right) for the absence/presence of the *G. balcanicus* species, for chemical oxygen demand (CCO-Cr)

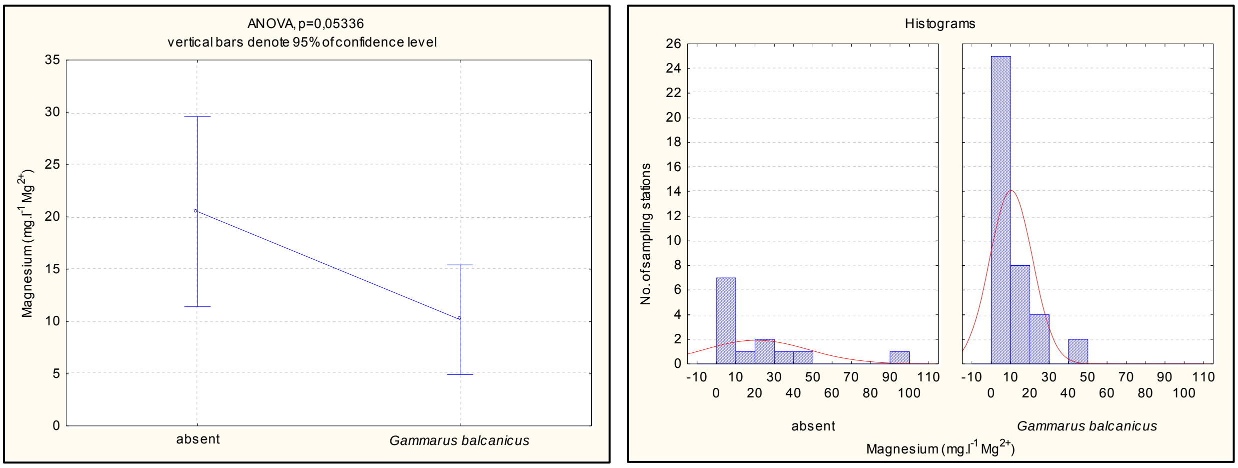


Figure 33: The ANOVA test (left) and the histograms (right) for the absence/presence of the *G. balcanicus* species, for magnesium

Very close to the confidence limit are also placed the results for the ammonia and for the anionic surfactants (figure 34, 35).

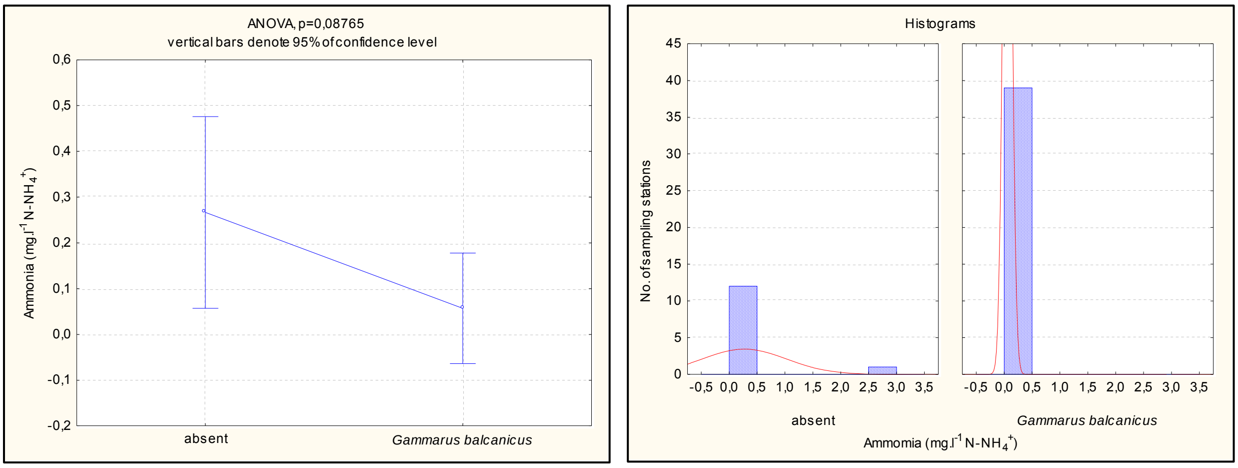


Figure 34: The ANOVA test (left) and the histograms (right) for the absence/presence of the *G. balcanicus* species, for ammonia

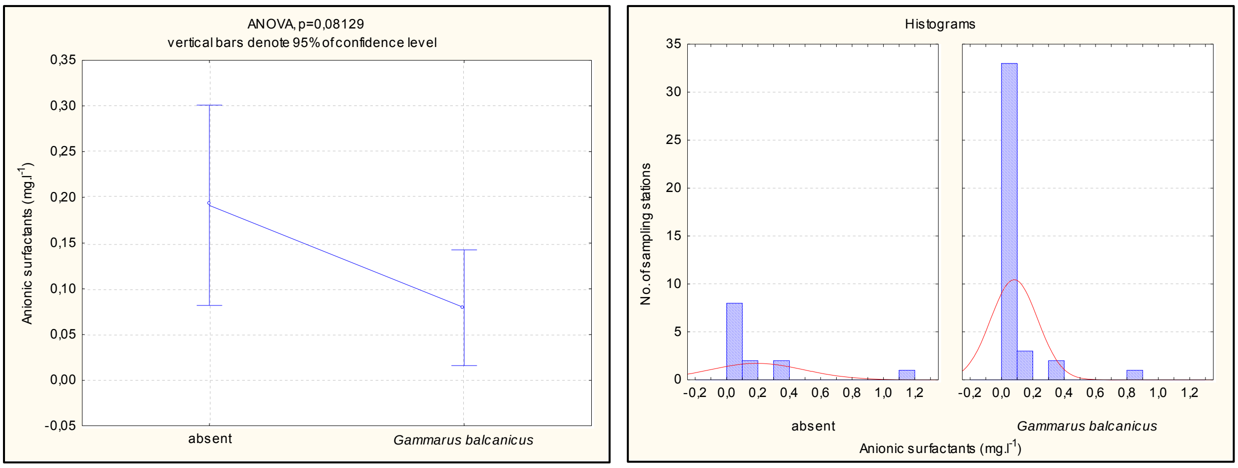


Figure 35: The ANOVA test (left) and the histograms (right) for the absence/presence of the *G. balcanicus* species, for anionic surfactants

In the case of the amphipod species *Gammarus fossarum* the statistic results have not passed the confidence threshold for none of the measured parameters. Similar values can be noticed in the case of complete hardness. The small number of stations in which this species has been identified makes the test to have a very low relevance level.

The relation between the chemical composition of the water and the isopod species *Asellus aquaticus*

This species prefers the aquatic habitats with a lower quality as compared to the other malacostraca species found in the Anina Mountains (Pashkova and Korotneva 2000, Hargeby 1990, Økland 1978). The statistic tests have surpassed the confidence threshold for 4 of the measured parameters: ammonia, nitrate and nitrite (figure 36-38).

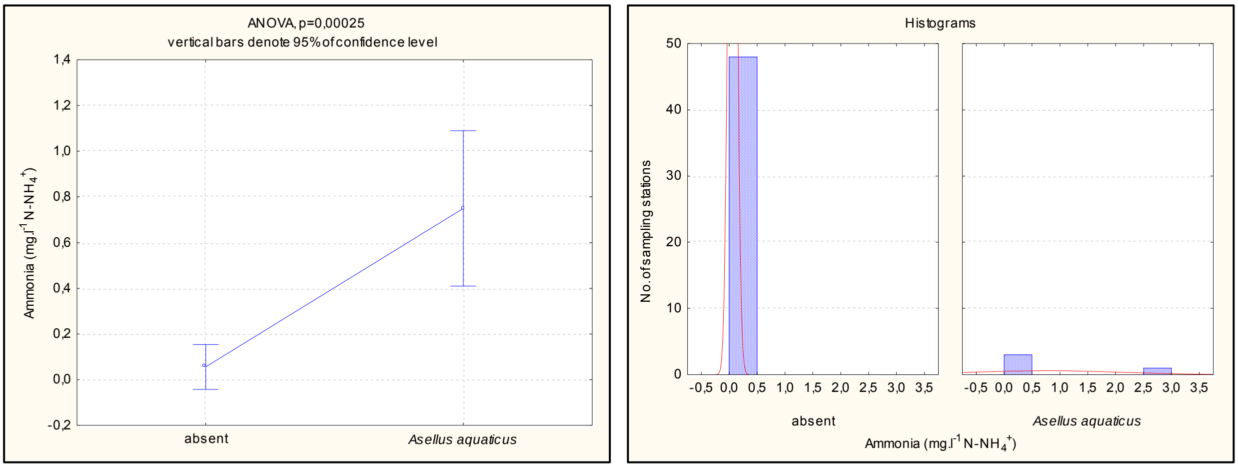


Figure 36: The ANOVA test (left) and the histograms (right) for the absence/presence of the *A. aquaticus* species, for anionic ammonia

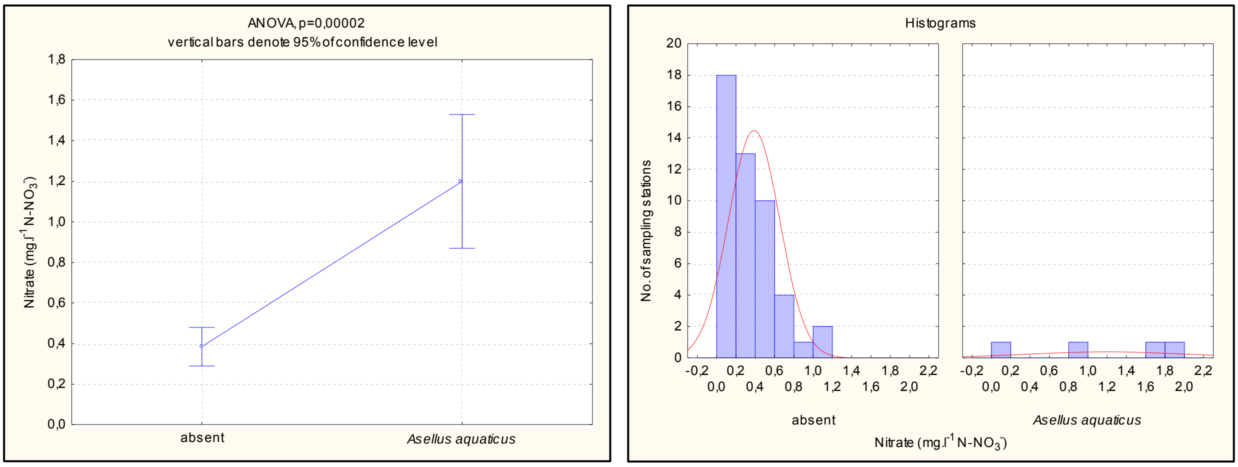


Figure 37: The ANOVA test (left) and the histograms (right) for the absence/presence of the *A. aquaticus* species, for nitrate

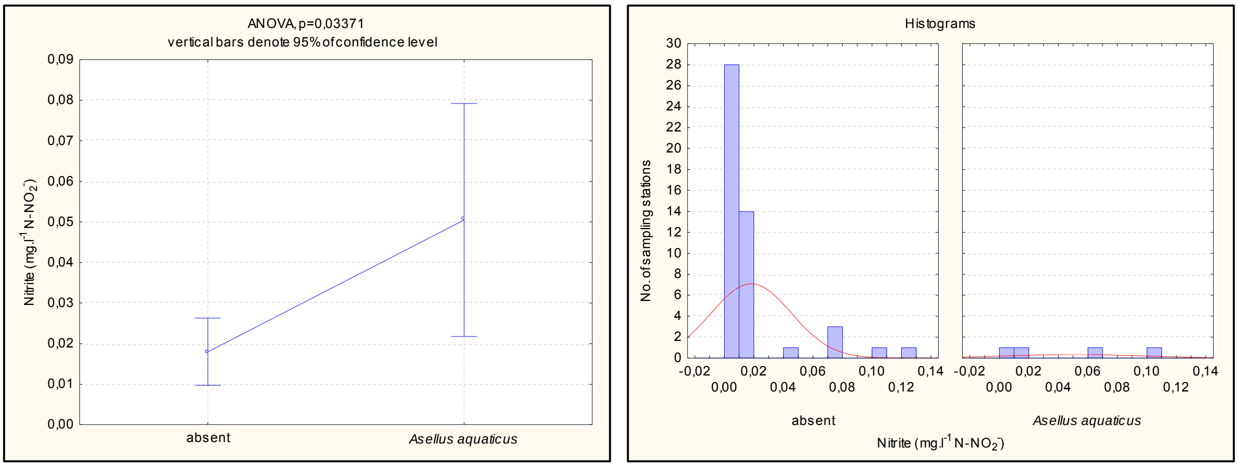


Figura 38: The ANOVA test (left) and the histograms (right) for the absence/presence of the *A. aquaticus* species, for nitrite

The statistic tests also show that the preferences of this isopod species for the water dissolved oxygen are not very high (figure 39).

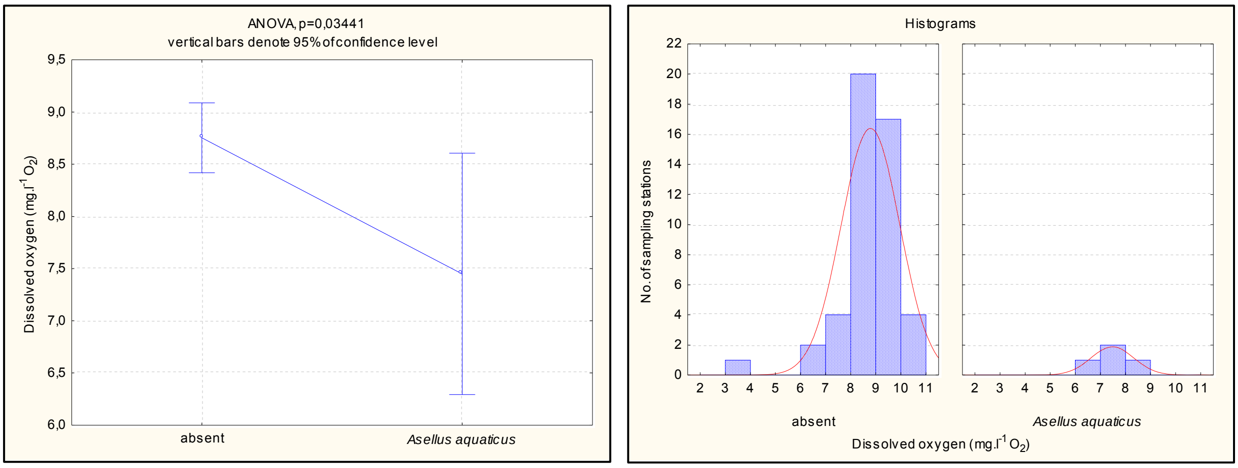


Figure 39: The ANOVA test (left) and the histograms (right) for the absence/presence of the *A. aquaticus* species, for dissolved oxygen

The anthropic impact

The main areas that may prove to have a negative impact on the water quality are the localities that have been built on a water course. Usually, the water quality falls in much inferior classes than the upstream class. The cities Reşiţa and Anina have the most significant impact on the rivers Bârzava and Gârlişte. It is the city Anina that also influences the quality of the brook Ştaier, although in a smaller amount through the Ştaierdorf quarter. Negative effects on the quality of the stream waters are also caused by rural settlements, the water of the brooks Clocotici and Cândeni being so polluted that it passes from a class II into a class V; in the case of Lăpuşnic and Moceriş the water quality falls into a class IV. On the main course of the river Nera the water is badly damaged by the upstream localities. At none of these stations decapod species have been discovered; the only species that has been discovered in these stations has been the isopod *Asellus aquaticus*.

A special situation has been encountered on the brook Cândeni, a brook that runs through the village Socolari, where with the occasion of the investigations we have conducted we have witnessed a mass mortality among the *A. torrentium* species, which was most probably caused by the traditional practice of doing the laundry, practice that is still very much in use in this village. Studying our in field observations we have also noticed a rise in the phosphate concentration at the Beiu station, placed at several tens of metres upstream the camping area Canton Beiu, a place that is highly populated by tourists during the summer, without representing a threat to the aquatic fauna on the long run.

A lowering of the water quality has also been noticed in areas where logging was taking place on the upstream; this lowering of the water quality was closely linked to the increase of the concentration of soluble reactive phosphorus. At the Liscov station the forest exploitation was in full development, the water quality falling into a class IV due to the presence of nitrites and into a class III due to the presence of soluble reactive phosphorus. An active forest exploitation has also been observed in the area upstream the brook Comarnic, where water falls into a class IV because of the soluble reactive phosphorus. However, the water quality of these stations does not damage significantly the malacostraca fauna although the upstream carrying of this compound may determine the eutrophy of the reservoirs. (DePinto *et al.* 1981, Sharpley 1993).

Negative effects concerning the lowering of the water quality can also be noted when brooks pass through artificial reservoirs; the Văliug lake being a relevant example. Here the water of the river Bârzava falls from a class III into a class IV; it no longer represents any malacostraca species for the benthic fauna; upstream it will return to a class III. In the case of smaller lakes it can be noticed that their water does not significantly alter their quality parameters. A negative effect on the water quality has been registered upstream of the brook Poneasca, an area in which works for the building of the new reservoir were conducted.

CONCLUSIONS

As a follow up of the investigations conducted on the aquatic malacostraca found in the lotic habitats of the Anina Mountains, we can draw the following conclusions:

- Two species belonging to the decapod order have been identified: the river crayfish – *Astacus astacus* (Linnaeus 1758) and the slope crayfish – *Austropotamobius torrentium* (Schrank 1803); three amphipod species: *Gammarus balcanicus* Schäferna 1922, *Gammarus fossarum* Koch, in Panzer 1835 and *Gammarus roeseli* Gervais 1835; one aquatic isopod species *Asellus* *aquaticus* (Linnaeus 1758). No species of invasive malacostraca have been identified in the waters of the Anina Mountains.

- The decapod *Austropotamobius torrentium* can be found in all of the three hydrographic basins, but its highest frequency turned out to be in the hydrographic basin of the Nera. The decapod *Astacus astacus* lives only in the Bârzava and Caraș basins, being most frequently found in the stations placed in the basin of the river Caraș.
- The most frequent amphipod species is *Gammarus balcanicus,* found in the waters of all of the three investigated basins. *Gammarus fossarum* lacks from the waters of the hydrographic basin of the river Nera. The *Gammarus roeseli* species has been found only in the samples collected from the Caraș basin, having a lower frequency than the other species of amphipods.
- The isopod *Asellus aquaticus* has been identified as having a low frequency in all of the samples collected from all of the three investigated basins.
- Most frequently species of malacostraca have been found in waters that have a swift type of running or a moderate one. Concerning the preference for a certain granulation of the soil, pebbles and gravel proved to be the ones mostly frequented by the species. The habitats found near water areas have predominantly been represented by deciduous forests and by mixed forests which ensure a high level of humidity. Altitude also plays an important part in the distribution of the *Astacus astacus*, *Gammarus balcanicus* and *Gammarus fossarum*.
- The statistic processing of the data concerning the distribution of the species, correlated with the values of the chemical parameters measured at each of the 52 investigated stations from the Anina Mountains, evidentiates the optimal level for each of the species.
    - The ammonia intolerance manifests itself only in the case of the *Gammarus balcanicus* while for the isopod *Asellus aquaticus* results show that the species tolerates relatively well even very high values.
    - Nitrate have a negative impact on the presence of the decapod *Austropotamobius torrentium,* but at the same time the *Asellus aquaticus* species manifests tolerance for high values.
    - The nitrite presence into the water is poorly tolerated by both decapod species, as well as by the amphipod *Gammarus balcanicus*, while the isopod *Asellus aquaticus* manifests a high level of tolerance for this parameter too.
    - Soluble reactive phosphorus do not influence significantly the distribution of decapods, but the amphipod *Gammarus balcanicus* has been proven to avoid the stations with a high phosphate concentration, although it has also been found at values that were close to the maximum limit.

- The level of dissolved oxygen has proven to be a determinant factor for the distribution of the decapod *Austropotamobius torrentium* and of the amphipod *Gammarus balcanicus*; these species prefer high values.

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