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THE RELATION BETWEEN WATER QUALITY AND THE DISTRIBUTION OF *GAMMARUS BALCANICUS* SCHÄFERNA 1922 (AMPHIPODA: GAMMARIDAE) IN THE ANINA MOUNTAINS

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Abstract: *Gammarus balcanius,* the commonest amphipod species in the mountainous and submountainous areas of Romania, has been the subject of various approaches regarding the relation between the species distribution and water quality. This paper compares by means of the one-way ANOVA test the data collected from the sampling stations where this species was detected with the data from the sampling stations where the species was not detected. Several parameters were taken into account: altitude, pH, dissolved oxygen, chemical oxygen demand, hardness, anionic surfactants, tannin, lignin, cyanide, nitrate, nitrite, ammonia and soluble reactive phosphorus. The preference of the species for a high level of dissolved oxygen, the low level of chemical oxygen demand, nitrite and soluble reactive phosphorus were the statistically significant results. The low levels of ammonia and anionic surfactants and the result regarding altitude are also close to the margin for statistical significance. All these results point to the conclusion that the species under scrutiny prefers the clean waters of the mountain streams' middle and superior segments.

Key words: Anina Mountains, Gammaridae, *Gammarus balcanicus,* distribution, water quality

1. INTRODUCTION

The majority of amphipod species reside in marine or brackish environments, but they also inhabit a wide range of freshwater habitats, in close relation to the environmental conditions (Ciubuc, 1985). Species diversity is higher in flowing waters, either cold, or subterranean, while the number of epigeal species is lower (Väinölä et al*.*, 2008). It has been demonstrated that human interventions that result in changes of the water stream morphology and configuration have a considerable negative impact for water quality (Gurzau et al., 2010) and also for crustaceans, which may even lead to the extinction of the affected populations (Lowery & Hogger, 1986; Pârvulescu, 2009a). As to their sensitivity to water quality, the gammarids are rated at 6 points in accordance with the Biological Monitoring Working Party Score system (Chapman et al., 1996).

The investigated area, i.e. the Anina Mountains, is located in south-western Romania and it includes two national parks: the Semenic-Cheile Carasului National Park and the Cheile Nerei-Beusnita National Park. The geology is predominantly calcareous (Bleahu & Rusu, 1965). This geographical unit is drained by three main water courses: the Bârzava, the Caraş and the Nera rivers. The Caraş and the Nera rivers are direct tributaries of the Danube River, while the waters of the Bârzava River reach the Danube only after the river's confluence with the Timiş (Ujvari et al., 1982). This area was strongly impacted by industrialization, in the form of dams, water-catchments, mining. Three amphipod species have been found in this area: *Gammarus balcanicus*, *Gammarus fossarum* and *Gammarus roeseli*; the widest spread species is *G. balcanicus* (Pârvulescu, 2009b).

The chemical oxygen demand (COD) test is commonly used to indirectly measure the amount of organic compounds in water. Most applications of COD determine the amount of organic pollutants found in surface water making COD a useful measure of water quality. Dissolved oxygen content was subject to concern, given the strong influence of the water quality indicators on the aquatic ecosystems; high mortalities of both invertebrates and fish are, most likely, the most dramatic effects of hypoxia in eutrophic and hypereutrophic aquatic ecosystems (Camargo & Alonso, 2006).

Pesticides have been shown to be detrimental to key groups of freshwater organisms, zooplankton richness, diversity, abundance and oxygen concentrations, all decreased in pulsed treatments (Alonso & Camargo, 2009), while phytoplankton and microbial abundance increased (Amy et al., 2008). The concentration of total ammonia consists of the sum of NH_4^+ (ionized ammonia) and unionized ammonia (NH_3), the equilibrium between the two forms being dependent on the pH and the temperature of the water: as the values of the pH and the water temperature tend to increase, the concentration of NH_3 also increases, while the concentration of NH_4^+ decreases. As far as the ammonia toxicity is concerned, it has been largely documented that unionized ammonia is very toxic to aquatic animals, whereas ionized ammonia is non-toxic or appreciable less toxic (Russo, 1985; Adams & Bealing, 1994; Richardson, 1997; Constable et al., 2003). According to US-EPA and other studies, on the basis of acute and chronic toxicity data, water quality criteria ranging 0.05–0.35 mg l^{-1} N-NH_3 for short-term exposures and 0.01–0.02 mg l^{-1} N-NH_3 for long-term exposures have been estimated and recommended to protect sensitive aquatic animals (US Environmental Protection Agency 1986 & 1999; Constable et al., 2003; Alonso & Camargo, 2004).

The main toxic action of nitrite on aquatic animals is due to the conversion of oxygen-carrying pigments into forms that are not able to carry oxygen, leading to hypoxia and eventually death (Tahon et al., 1988; Jensen, 2003; Camargo & Alonso, 2006). On the basis of acute toxicity data, (Alonso & Camargo, 2003) estimated water quality criteria in the range 0.08–0.35 mg l^{-1} N-NO_2 as being adequate to protect sensitive aquatic animals, at least during short-term exposures.

The toxicity of nitrate ions in aquatic ecosystems has been traditionally considered as irrelevant (Russo, 1985; Camargo et al., 2005), even though the 25 mg l^{-1} N-NO_3 level can be reached in surface waters. Similarly to nitrite, the main toxic action of the nitrate ion (NO_3) relates to the conversion of oxygen-carrying pigments into forms that are incapable of carrying oxygen. The most important characteristic of nitrate behaviour is actually the fact that before it becomes toxic, nitrate must be converted into nitrite under internal body conditions (Cheng & Chen, 2002). However, when compared to nitrite's toxicity, it is worth mentioning that due to the low branchial permeability of the nitrate ions, the NO_3 uptake in aquatic animals is more limited than the NO_2 uptake, which contributes to the relatively low toxicity of nitrate (Jensen, 1996; Scott & Crunkilton, 2000; Cheng & Chen, 2002; Alonso & Camargo, 2003; Camargo et al., 2005). Moreover, several laboratory studies have shown that a nitrate concentration of 10mg. l^{-1}N-NO_3 (which is the USA federal maximum level for drinking water) can adversely affect, at least during long-term exposures, sensitive aquatic animals (Canadian Council of Ministers of the Environment 1991, Camargo et al., 2005). On the basis of the toxicity data, the Canadian Council of Ministers of the Environment (2003) recommended a water quality criteria range of 2.9–3.6 mg l^{-1}N-NO_3 to protect freshwater and marine life, while Camargo et al. (2005) proposed a maximum level of 2 mg l^{-1}N-NO_3 for the protection of sensitive aquatic animals.

Hydrogen cyanide and other cyano-compounds that liberate free cyanide ions are highly toxic to almost all forms of fauna (Donato et al., 2007). In biota, the cyanide binds to iron; copper and sulfur-containing enzymes and proteins required for oxygen transportation to cells, in higher animals, the major affected organ is the brain, with a decrease in cell function leading to coma and collapse of the respiratory and cardiovascular systems (Ballantyne, 1987).

Calcium carbonate is a vital component for the formation of crust in aquatic crustaceans. Laboratory tests show that hatching egg in some fish species depend on water hardness (Molokwu & Okpokwasili, 2002). Also, water hardness had a significant effect on heavy metals toxicity. The concentrations of metals necessary to immobilize 50% of the test animals at 24, 48, 72, 96 hr were significantly different in soft and hard water (Rathore & Khangarot, 2002). The concentration of the surfactant in the liquid depends, in turn, upon the interaction between surfactant and water hardness ions, as well as the electrolyte concentration (Hu & Tuvell, 1988).

The harmful effects of surfactants to the environment are well known. Considerable amounts of surfactants are being released in wastewaters and the negative effect of these toxicants could significantly affect the role of P-accumulating bacteria, as well as eukaryotic organisms (Hrenovic & Ivancovic, 2007). In some situations, excessive concentration released by the crossing of settlements can cause serious damage among aquatic fauna (Pârvulescu, 2009a).

2. MATERIALS AND METHODS

2.1 Sampling sites and data collection.

Biologic samples were collected between July-August 2008 and repeated in August 2009, in 52 sampling stations, on all the permanent waters in the upper sector of the Bârzava, Caraş and Nera rivers. Most of these sampling stations are located within two National Parks (Semenic-Cheile Caraşului National Park and Cheile Nerei-Beuşniţa National Park). Each sampling station was comprised of at least 50 m of river under investigation. On this occasion, observations were carried out with regard to the riverbed morphology and the surrounding habitats. For collecting the amphipods we used a Surber sampler with a catchment area of 300 cm^2 and a mesh size of 350 µm, followed by further research among solid objects and plants on the bottom of the river. The collecting surface was established at five randomly chosen different squares per sampling station, on the river sector, for covering more microhabitats.

Several general physicochemical indicators were measured in each of the 52 sampling stations: water temperature, pH, dissolved oxygen (DO), hardness (Hrd), anionic surfactants (AS), tannin+lignin (Tan+Lig), cyanide (Cyan), chemical oxygen demand (COD), dissolved inorganic nitrogen forms (N-nitrate, N-nitrite, N-ammonia) and soluble reactive phosphorous (SRP). These indicators were recorded by *in-situ* measurements with HACH Lange DR field equipment.

2.2 Statistical analysis.

Once the individuals captured on the river sector under investigation were identified, the dates were employed in statistical analyses. To perform the statistical analysis, we used the STATISTICA software version 7.0 for Windows (StatSoft Inc.). The estimated parameters were: the mean, minimum and maximum values and standard deviation. One-way ANOVA test were applied for every parameter in order to reveal differences between presence and absence of the species.

3. RESULTS AND DISCUSSION

Based on the findings of the 52 sampling stations dispersed over all the permanent rivers in the Anina Mountains, we were able to create an image of the epigean species distribution. In the three water basins of the Anina Mountains the *G. balcanicus* was identified in all basins, while *G. fossarum* was completely absent from the Nera basin and *G. roeseli* was found only in the Caraş basin (Pârvulescu, 2009b).

In order to find a better link between the water quality (as given by the selected physico-chemical indicators) and the absence/presence of *G. balcanicus* species, the obtained data sets were subject to ANOVA analysis, between the set of data from the sampling stations where the *G. balcanicus* species was found and the set of data from the sampling station where the species was not found. The diagrams which show the frequency (figures 1 to 8) emphasize the interval of values for the presence or the absence of this species.

It is difficult to identify a concrete link between *G. balcanicus* species distribution and qualitative water parameters, because the range of colonised habitats depends on the natural environment heterogeneity within study areas (Legalle et al., 2008). According to the ANOVA test certain parameters have a decisive effect on the distribution of the *G. balcanicus* amphipod.

Table 1. Variation ranges and ANOVA tests for the selected physico-chemical indicators, measured in 2009 in the Anina Mountains Rivers under investigation (significant ANOVA p values in bold).(number of stations = 52)

Variable (unit)	ANOVA (p value)	Min - max	Mean ± SD
Altitude (m)	0.09155	190 - 900	402.17 ± 161.86
pH	0.81925	7.24 – 8.48	7.91 ± 0.33
Dissolved oxygen (mg l^{-1} O_2)	**0.01486**	3.13 – 10.13	8.65 ± 1.19
Hardness (°dH)	0.86806	0.69 – 28.7	8.65 ± 5.8
Anionic surfactants (mg l^{-1})	0.08129	0.009 – 0.871	0.107 ± 0.2
Tannin+lignin (mg l^{-1})	0.91260	0.1 – 2.3	0.65 ± 0.5
COD (mg l^{-1} O_2)	**0.03745**	2.5 – 5.8	3.4 ± 1.7
Cyanide (mg l^{-1})	0.84114	0.001 – 0.019	0.005 ± 0.003
Nitrate (mg l^{-1} N-NO_3^-)	0.12531	0.1 – 1.9	0.447 ± 0.392
Nitrite (mg l^{-1} N-NO_2^-)	**0.02341**	0.001 – 0.124	0.02 ± 0.029
Ammonia (mg l^{-1} N-NH_4^+)	0.08765	0.007 – 2.78	0.109 ± 0.383
SRP (mg l^{-1} P-PO_4^{3-})	**0.03816**	0.02 – 1.27	0.249 ± 0.284

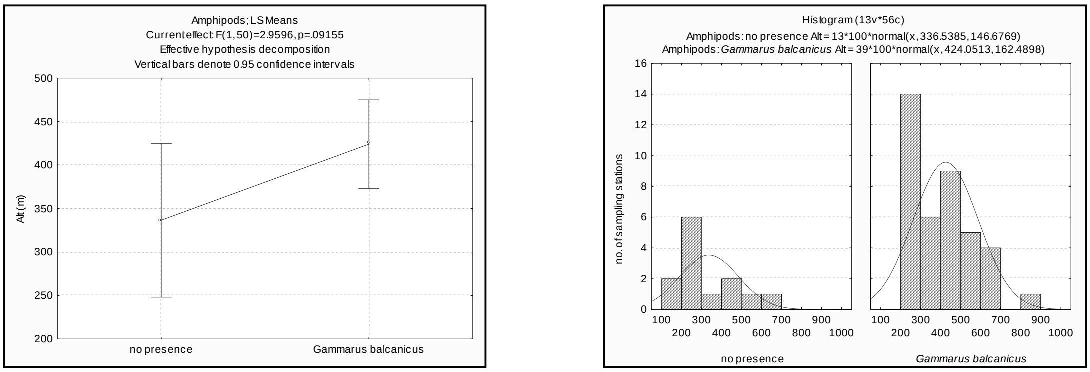

Figure 1. The ANOVA test (left) and the frequency histograms of sampling stations (right) with and without *G. balcanicus*, for altitude (Alt)

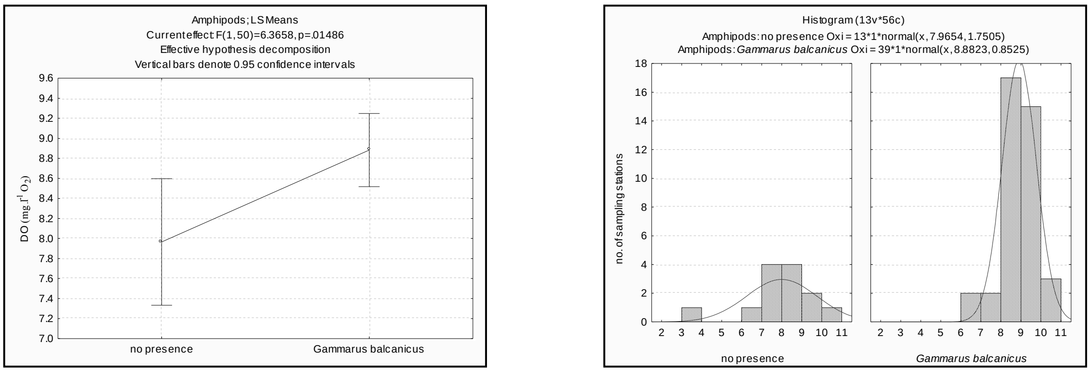

Figure 2. The ANOVA test (left) and the frequency histograms of sampling stations (right) with and without *G. balcanicus*, for dissolved oxygen (DO)

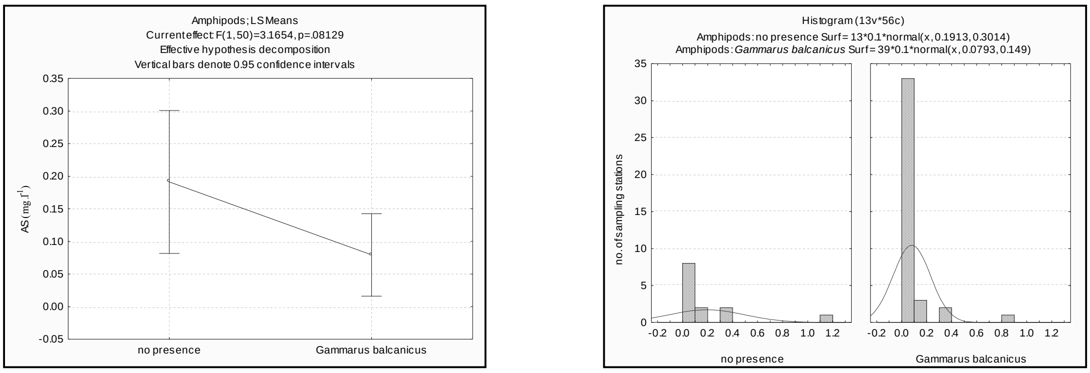

Figure 3. The ANOVA test (left) and the frequency histograms of sampling stations (right) with and without *G. balcanicus*, for anionic surfactants (AS)

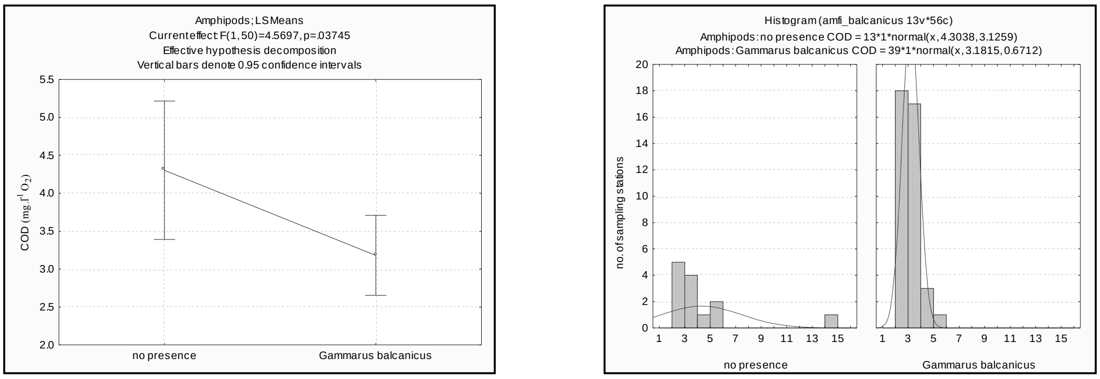

Figure 4. The ANOVA test (left) and the frequency histograms of sampling stations (right) with and without *G. balcanicus*, for chemical oxygen demand (COD)

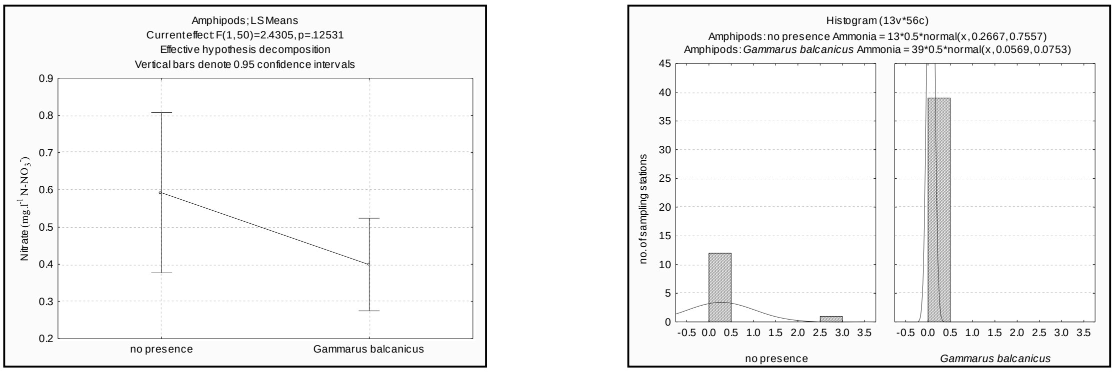

Figure 5. The ANOVA test (left) and the frequency histograms of sampling stations (right) with and without *G. balcanicus*, for nitrate

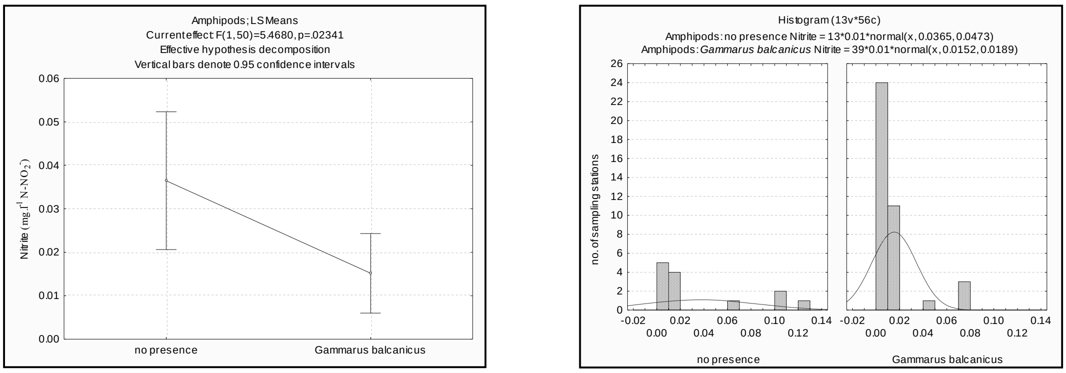

Figure 6. The ANOVA test (left) and the frequency histograms of sampling stations (right) with and without *G. balcanicus*, for nitrite

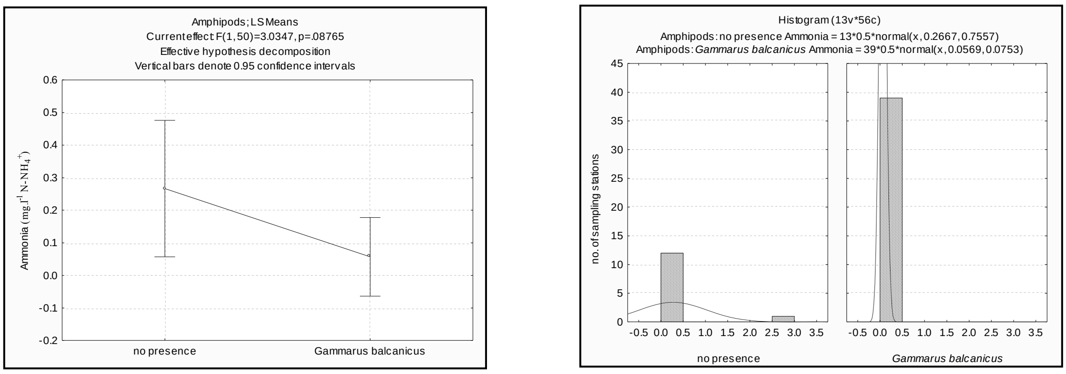

Figure 7. The ANOVA test (left) and the frequency histograms of sampling stations (right) with and without *G. balcanicus*, for ammonia

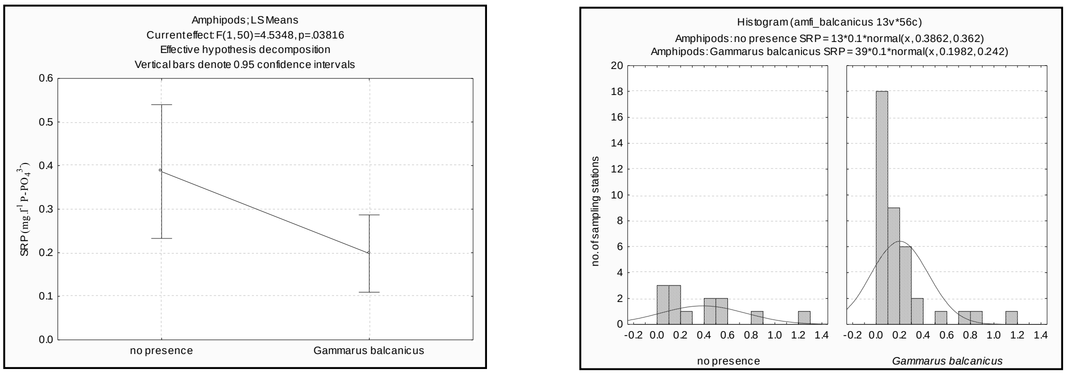

Figure 8. The ANOVA test (left) and the frequency histograms of sampling stations (right) with and without *G. balcanicus*, for soluble reactive phosphorus (SRP)

One can say that the following parameters are limitative factors for the distribution of the *G. balcanicus* species: dissolved oxygen, chemical oxygen demand, nitrite and soluble reactive phosphorus.

Figure 2 and figure 4 show that in most of the sampling stations in which *G. balcanicus* was found, dissolved oxygen and chemical oxygen demand ranged in the intervals 6.29 – 10.13 and 2.5 – 3.8mgl^{-1} O_2 respectively, which indicates almost no organic pollution. This situation leads to the conclusion that *G. balcanicus* prefers clean well-oxygenated waters. As far as nitrite is concerned, the results show the intolerance of *G. balcanicus* towards this chemical; most of the specimens were collected in stations where the nitrite concentration was lower than 0.02 mg l^{-1}, even if several specimens were collected at a level of 0.08 mg l^{-1} N-NO_2 (figure 6). For soluble reactive phosphorus (SRP), results indicate that most of *G. balcanicus* specimens were collected in sampling stations in which concentrations were below 0.16 mg l^{-1} P-PO_4^3 and there was no presence of this species in stations with concentrations in the level of SRP at 0.32 – 0.48 mg l^{-1} P-PO_4^3 (figure 8). As far as the altitude is concerned, the sampling stations were situated in the interval 190-900 m; the ANOVA test showed that the species prefers the medium and high altitudes (figure 1).

The results concerning the anionic surfactants (figure 3) which were near the relevance margin of the ANOVA test are not significant because the sampling area is a mountainous region where most of the waters are hardly affected by the anthropical factor, except the stations situated downstream of human settlements. As far as the nitrate is concerned, the results obtained in the Anina Mountains are not significant for the distribution of *G. balcanicus*; however, the species displayed a preference for levels below 0.6 mg l^{-1} N-NO_3, even if specimens were found at levels of 1.9 mg l^{-1} N-NO_3 (figure 5). Similarly, for N-ammonia, the amphipod was found at levels below 0.5 mg l^{-1} N-NH_4 (figure 7).

There was no evidence for a preferential distribution of *G. balcanicus* as far as the following parameters are concerned: pH (measured in the interval 7.24 – 8.48), water hardness (0.7 – 28.7 ^{0}dH), tannin + lignin (most of the concentrations were below 0.5 mg l^{-1}), and cyanide (measured in the interval 0.001 – 0.019 mg l^{-1}).

5. CONCLUSIONS

- The ecological preferences which are important for the occurrence of the *Gammarus balcanicus* amphipod species are the high level of dissolved oxygen and the low levels of chemical oxygen demand and nitrate. Values which are close to the relevance limit were obtained for anionic surfactant (extremely important for water quality, but irrelevant in the area under scrutiny because of the low percentage in this mountainous area), nitrate, and ammonia.
- Altitude is close to the statistic relevance of the ANOVA test, underlying the ecological preferences of *Gammarus balcanicus* for waters situated at the level where the anthropical impact is low.
- The ANOVA statistical tests show that pH, hardness, tannin and lignin, and cyanide are irrelevant for the measured intervals in Anina M-ts.

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