# EFFECTS OF THE ENVIRONMENTAL VARIABLES ON THE ALIEN AMPHIPOD *PONTOGAMMARUS ROBUSTOIDES* IN THE DAUGAVA RIVER AND ITS RESERVOIRS

**Jana Paidere, Aija Brakovska, Vasil Vezhnavets, Artūrs Škute, Marina Savicka**

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The Ponto-Caspian *Pontogammarus robustoides* is the dominant alien amphipod in Latvian inland waters, in particular it mostly occurs in the Lower Daugava River. Effects of the environmental variables (water level, physico-chemical parameters and season) on the population structure of alien amphipod *P. robustoides* in the Daugava River and its reservoirs was done in 2016 from May to September. The results showed that the greatest impact on the alien amphipod *P. robustoides* population structure, particularly on immature individuals is caused by the reservoir eutrophic conditions and water level. The immature individuals can be more herbivore and prefer habitats with lower water level. If nutrient levels or trophy, specifically phosphorus increase in the Daugava River in the future, the higher invasion success is to be expected.

Key words: alien amphipod, *Pontogammarus robustoides*, environmental variables, Daugava River, reservoirs.

*Jana Paidere, Aija Brakovska. Laboratory of Hydroecology, Department of Ecology, Institute of Life Sciences and Technologies, Daugavpils University, Daugavpils, Parādes 1a, Latvia,E-mail: jana.paidere@du.lv, aija.brakovska@inbox.lv*
*Vasil Vezhnavets. Scientific and Practical Center for Bioresources of the National Academy of Sciences of Belarus, Minsk, Akademycheskaya St 27, Belarus, E-mail: vvv@biobel.bas-net.by*
*Artūrs Škute, Department of Ecology, Institute of Life Sciences and Technologies, Daugavpils University, Daugavpils, Parādes 1a, Latvia, E-mail: arturs.skute@du.lv*
*Marina Savicka, Laboratory of Molecular Biology and Genetics, Department of Ecology, Institute of Life Sciences and Technologies, Daugavpils University, Daugavpils, Parādes 1a, Latvia, E-mail: marina.savicka@du.lv*

## INTRODUCTION

Important stressors in freshwater ecosystems are identified land use, eutrophication, habitat destruction and biological invasions (Dudgeon et al. 2006, Stendera et al. 2012). Attention to alterations of the hydrological regime is also devoted. River transformation into slow current water bodies due to impoundment and construction reservoirs or navigable waterways promote environmental changes (variability in water levels and water physico-chemical parameters, water quality degradation, and habitat destruction) and thereby allow identification of environment that either facilitate or inhibit establishment and spread processes of biological invasion (Havel et al. 2005, Johnson et al. 2008, Hänfling et al. 2011, Ricciardi, MacIsaac 2011, Früh et al. 2012).

Between alien peracaridan crustaceans amphipods, especially Ponto-Caspian, are one of the most successful invaders in fresh and brackish European waters (Bij de Vaate et al. 2002, Grabowski et al. 2007, Berezina et al. 2011, Semenchenko et al. 2015, Arbačiauskas et al. 2017), mainly due to created cascades of reservoirs, canalisation and navigable waterways, and due to intentional introduction of alien species into reservoirs (Jażdżewski, Grabowski 2011, Leuven et al. 2009, Semenchenko et al. 2015). In Latvian inland waters, such successful invader is Ponto-Caspian gammarid *P. robustoides*. Currently, *P. robustoides* has occurred and has become the dominant gammarid in the reservoirs of the Daugava River (Pļaviņas, Riga, Ķegums) (Paidere et al. 2016), as well as has occurred and is the most numerous in the Lake Liepāja, the Lake Ķīšezers, and the estuaries of Daugava and Venta rivers, and has occurred in river mouths (Gauja, Lielupe, Salaca, Saka) that flow into the Baltic Sea (Grudule et al. 2007). Thereby, it points out different introduction routes of the species in Latvian inland waters. Firstly, a Ponto-Caspian gammarid *P. robustoides* initially was intentionally introduced in Latvian inland waters as valuable fish food in the Ķegums Reservoir of the Lower Daugava River and the nearest lake (the Lake Lielais Baltezers) to Riga in the 1960s (Kachalova, Lagzdin 1968, Bodniece 1976). Secondly, penetration of *P. robustoides* in the lower reaches and river mouths that flow into the Baltic Sea and in the onshore lakes of the sea can be explained by the introduction of species to Neman River and/or Latvian inland waters and further expansion on Curonian Lagoon and on the coastal waters of the Baltic Sea (Jażdżewski, Jażdżewski 2008, Arbačiauskas et al. 2017).

Results from a recent study of the occurrence and frequency of *P. robustoides* by different size and habitats in the reservoirs of the Daugava River provide evidence that very slow or stagnant and shallow waters with different habitats are favourable for the species, and *P. robustoides* occurrence by different body size within both filamentous algae and macrophytes is significant and could be explained by the feeding behaviour of species in different individual development stages (juveniles and adults) and by reduced competition and predators (Paidere et al. 2016). Thus, further research aim of continued *P. robustoides* studies was to determine the environmental variables (hydrological and water physico-chemical) that are favourable for the establishment of alien amphipod *P. robustoides* in the Daugava River and its reservoirs. Moreover, recent studies note that environmental conditions play an important role in the spread and establishment of reservoirs by alien species. The studies using stable isotope analysis in the Lithuanian eutrophic Curonian Lagoon of Baltic Sea and mesotrophic Lake Plateliai as well as in Russian eutrophic Neva Estuary of Baltic Sea showed trophic position or feeding strategy in the aquatic food chain of alien amphipods. They showed that trophic position of alien amphipods (*P. robustoides, Obesogammarus crassus* and other) varied significantly within different nutrient status of environments among species and during their ontogenesis. Juvenile stage or small size individuals of *P. robustoides* are characterised as herbivores/detritivores in both eutrophic and mesotrophic conditions, but adults or large body individuals are omnivores or carnivores in the more eutrophic conditions (Berezina et al. 2017, Arbačiauskas et al. 2013).

## MATERIAL AND METHODS

The Daugava River is one of the largest rivers in Eastern Europe. Starting in the Valday Highlands in Russia, the river flows through the East-European Plain and crosses Belarus and Latvia before flowing into the Gulf of Riga. The catchment area of the Daugava River is around 87 900 km$^2$ and its total length is around 1005 km, of which 342 km are located in Latvia. The cascade of three large hydroelectric power plants at Pļaviņas, Ķegums and Riga on the Lower Daugava creates a regulated run-of-river

Table 1. Characteristics of the Daugava reservoirs.

| Characteristics | Pļaviņas Reservoir[1] | Ķegums Reservoir[2] | Riga Reservoir[3] |
|---|---|---|---|
| Volume, million $m^3$ | 509.5 | 157 | 300 |
| Area, $km^2$ | 35 | 24.9 | 40 |
| Mean depth, m | 14.5 | 6.3 | 7.1 |
| Maximum depth, m | 47 | 16.5 | 17.4 |
| Reservoir length, km | 45 | 22 | 35 |
| Reservoir maximal width, km | ~ 2 | ~ 1.4 | ~ 4 |
| [4] Mean annual discharge, $m^3$ $s^{-1}$ | 567 | 575 | 590 |

[1]Tidriķis 1997, [2]Placēna 1995, [3]Lūmane 1997, [4]Projektējamās jaunās celulozes rūpnīcas notekūdeņu ietekmes ... 1998.

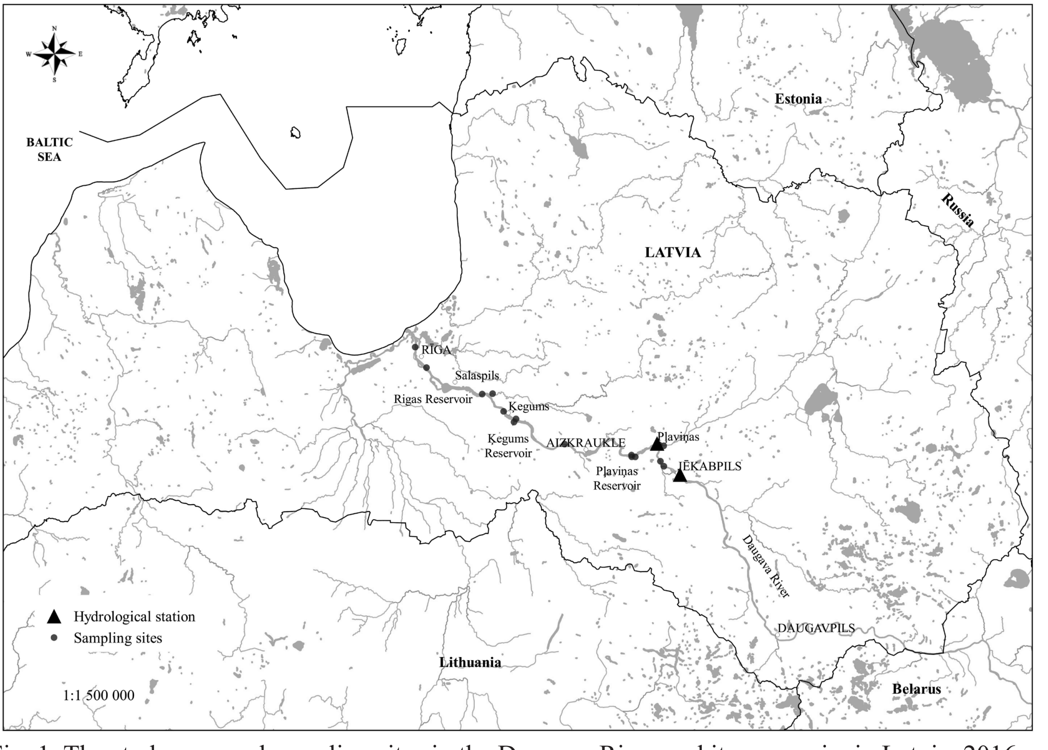


Fig. 1. The study area and sampling sites in the Daugava River and its reservoirs in Latvia, 2016.

hydrological regime and forms the largest artificial river reservoirs in Latvia (Volchak, Lyakmund 2006). Due to operation of hydropower plants, the water level is impacted not only in the reservoirs, but also in the upper stretches of the Daugava River. Some characteristics of the Daugava reservoirs are shown in Table 1. Reservoirs are characterised as eutrophic (Daugavas ekoloģiskā stāvokļa novērtējums 2007). Also, measurements of Secchi disk during this research in deepest part of Ķegums (in July), Pļaviņas (in July and in August) and Riga (in July) reservoirs have corresponded to eutrophic waters, the water transparency was 1.30, 1.40 and 0.97, and 1.20 m respectively, and the nutrients (mean $N_{tot}$ and $P_{tot}$ in the 2016 year) in the reservoirs of the

Pļaviņas, Ķegums and Riga, and the Daugava River at Riga was 1.2 and 0.078, 1.08 and 0.071, 1.65 and 0.079, 1.67 and 0.073 mg $l^{-1}$ respectively (Pārskats par Latvijas virszemes un …. 2017).

The research was done in 2016 in the lower parts of the Daugava River in Riga, Ķegums, and Pļaviņas reservoirs as well as in the upstream to Pļaviņas Reservoir and the downstream to Riga Reservoir at Riga. The research covered both the seasonal studies in the Pļaviņas Reservoir and the one-off studies in the Daugava River and its reservoirs. Seasonally, the samples were obtained from May until September once a month in the Pļaviņas Reservoir and upstream of the Pļaviņas Reservoir in September. In July the sampling was carried out in the Ķegums Reservoir and the upstream from the reservoir at Klidziņa, in the Riga Reservoir and the downstream of the Daugava River at Riga (Fig. 1).

The physico-chemical water parameters: water temperature °C, conductivity µS $cm^{-1}$, dissolved oxygen mg $l^{-1}$, pH, redox potential mV and chlorophyll α µg $l^{-1}$ were measured in the near-shore zones of the reservoirs/river in the water surface ± 1 m limits using a HACH DS5 multiprobe. Additional information on water level fluctuation data in the territory of research was obtained from data bases available in Latvian Environment, Geology and Meteorology Centre. Geographical data were obtained by echo sounder with GPS receiver LOWRANCE LMS-522C.

The substrate and vegetation in sampling sites consisted mainly of sand with different sized cobbles and boulders covered by mud and detritus, and by emerged and submerged macrophytes *Acorus calamus*, *Carex* sp., *Typha* sp., *Scirpus lacustris*, *Phragmites australis*, *Nuphar lutea*, *Potamogeton* sp.

Qualitative samples of amphipods were obtained by hand net with a mouth opening of 25 x 25 cm (500 µm) in the wadeable (up to 0.5 m) depths of the river and reservoirs. The final sample consisted of 10-12 sweep units. Samples were preserved in 75% ethanol. Identification of individuals was done using the following literature: Eggers, Martens 2001, Eggers & Martens 2004, Guide for Identification of the Fauna of the Black and Azov Seas 1969. Length of individuals was measured with ZEISS Stemi 2000 stereomicroscope. The length of the individuals was measured as a distance from the anterior margin of the head to the posterior margin of the telson (Bacela, Konopacka 2005). Individuals were divided by body length in three groups: small (< 5 mm), medium (5 – 8 mm), and large (> 8 mm).

Redundancy Analysis (RDA) was done to test the importance of environmental variables structuring *P. robustoides* population by a body length in the study area. The body length and environmental variables were logarithmically transformed before analysis. A multivariate analysis was done using Canoco 4.5 (Lepš, Šmilauer 2003). With an aim to find a significant relationship between the body length and water level changes in the Pļaviņas Reservoir, the Spearman rank correlation was done using IBM SPSS Statistics 20 (Dytham 2011).

## RESULTS

During the period of seasonal research in the Pļaviņas Reservoir, the changes of water level depended from the hydrological regime of the Daugava River and the operation of the Pļaviņas Hydropower Plant. It was observed that with the higher water level in the Daugava River the mean diurnal water changes in the Pļaviņas Reservoir were higher especially in August whilst it was the lowest water level in summer (Fig. 2).

Seasonally the water physico-chemical parameters were varied mainly according to the hydrological and meteorological season. For example, water conductivity continuously increased from spring (May, June) mean 287, 275 µS $cm^{-1}$ respectively to 382 µS $cm^{-1}$ later summer (September) in the Pļaviņas Reservoir. In addition, other water physico-chemical parameters were mainly changing seasonally, except for temperature in May when the average air temperature was 3.4° C above mean May temperature norm in

Table 2.Environmental parameters in the study area and time (mean ± standard deviation (SD)).

| Season | Sites | Relative water level, m mean daily m$^{-1}$ | Temperature, °C | Dissolved oxygen, mg l$^{-1}$ | pH | Oxred potential, mV | Chlorophyll α, µg l$^{-1}$ |
|---|---|---|---|---|---|---|---|
| May | Pļaviņas Reservoir | 9.24±0.10 | 20.0±1.86 | 9.59±1.22 | 7.90±0.32 | 333±19 | 7.23±3.10 |
| June | | 9.37±0.12 | 19.2±0.51 | 5.96±0.98 | 7.50±0.22 | 420±22 | 7.44±2.11 |
| July | | 9.32±0.15 | 21.6±0.56 | 7.84±0.38 | 8.16±0.21 | 383±15 | 9.44±2.80 |
| August | | 8.96±0.11 | 20.1±1.61 | 8.93±0.83 | 8.28±0.29 | 427±5 | 9.23±2.83 |
| September | | 9.67±0.14 | 18.6±0.04 | 8.10±0.03 | 8.29±0.01 | 444±2 | 5.45±0.12 |
| July | Ķegums Reservoir | - | 20.4±0.43 | 6.42±0.24 | 7.71±0.04 | 387±13 | 3.41±0.31 |
| | Riga Reservoir | - | 19.7±0.17 | 6.59±0.22 | 7.72±0.04 | 411±4 | 2.67±0.02 |
| | Daugava River (Riga) | - | 20.7±0.05 | 6.82±0.10 | 7.80±0.02 | 400±2 | 6.40±0.03 |

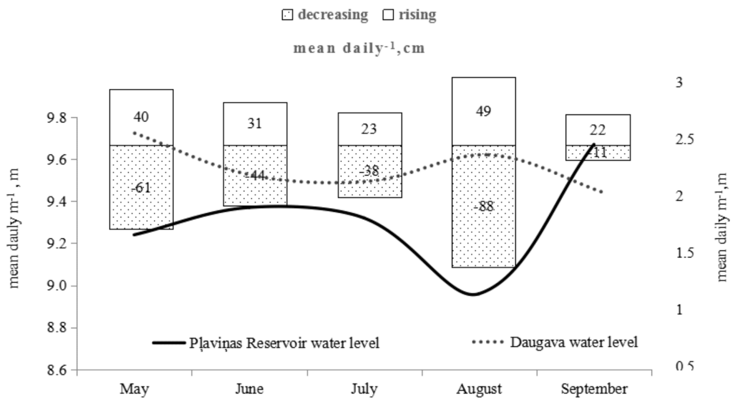


Fig. 2. Seasonal changes of the water level in the Daugava River (the hydrological station at Jēkabpils) and the Pļaviņas Reservoir (the hydrological station at Pļaviņas).

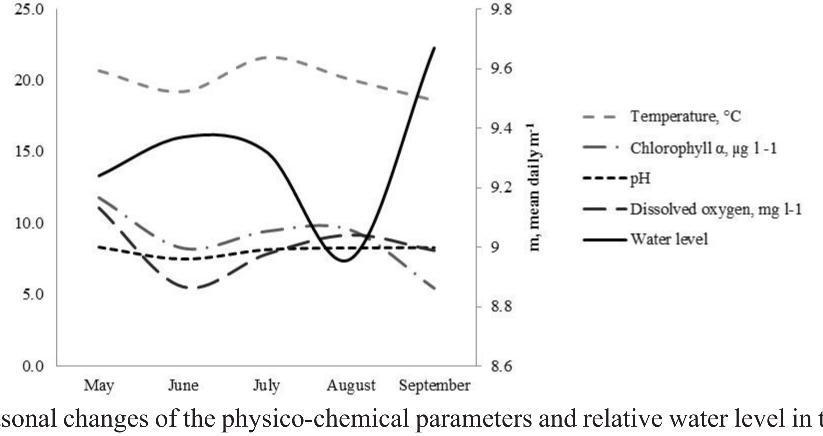


Fig. 3. Seasonal changes of the physico-chemical parameters and relative water level in the Pļaviņas Reservoir.

Latvia. May was characterised as a warm and dry month with atypical hot days. Thereby, the high values of water temperature, dissolved oxygen and chlorophyll α concentrations were observed in May. The negative relationship between the changes in the water level of the Pļaviņas Reservoir and dissolved oxygen, temperature, and chlorophyll α concentrations was obtained, but it was not statistically significant (Table 2, Fig. 3).

In July the physico-chemical parameters of the Ķegums and the Riga reservoirs and in September in the Daugava River also mainly corresponded to the season (Table 2).

*Pontogammarus robustoides* occurred in all reservoirs of the Daugava River, indigenous or other alien amphipods were not found there, except for the Ponto-Caspian amphipod *Obesogammarus crassus* which was found in the Daugava River aquatorium of the port Riga at the site at Voleri.

During the period of seasonal research, the individuals of *P.robustoides* were obtained in all months from May till September. Overall, populations of *P.robustoides* consisted of individuals with size from 2.0 to 16.5 mm (Fig. 4). Large or adult individuals were observed from May to September. In May larger individuals occurred with very low frequency (mean size 15.5 mm ± 0.65 standard deviation SD), then the length of the large individuals decreased. In August and September, the length increased again. Small or juvenile individuals were observed from June to September with a mean body length 3.8 mm ± 0.78 SD, but with very low frequency in September. The medium size individuals were observed fairly regularly from June to September with a mean body length of 6.7 mm ± 0.81 SD (Fig. 4).

Correlation between the groups by body length and the water level of the Pļaviņas Reservoir showed that the group of medium size individuals (5 – 8 mm) negatively and significantly correlated to the water level ($r = -0.73$, $p < 0.026$).

According to Monte Carlo permutation test, chlorophyll α concentration, pH values and oxred potential were identified as significant variables (pH, $p<0.01$, chlorophyll α, $p<0.03$ and oxred potential, $p<0.05$) which determinate population size structure in the study area (Fig. 5). The first axis explains 62% and the second axis 37% of the variance of the data set, and 54% of the total variance explains with the first two axes. Oxred potential (positively) and chlorophyll α, and pH (negatively) associated with the first axis, and only pH (negatively) associated with the second

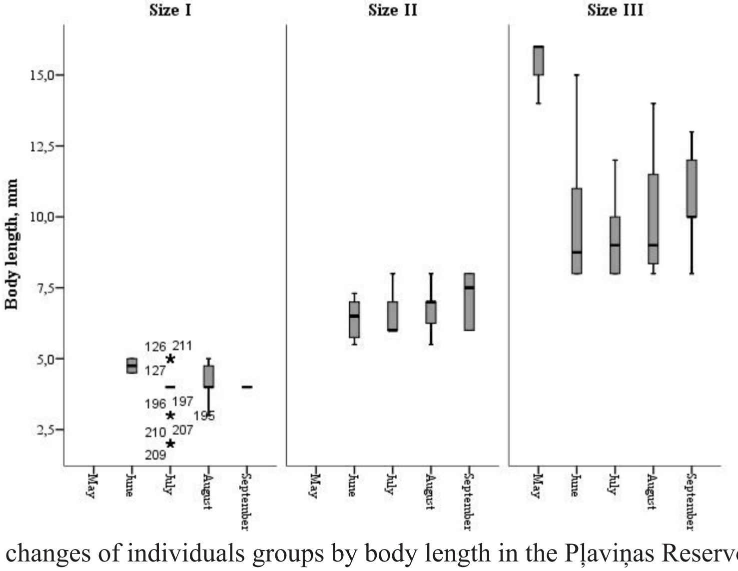


Fig. 4. Seasonal changes of individuals groups by body length in the Pļaviņas Reservoir.

axis. The higher values of chlorophyll α and pH was observed in the Pļaviņas Reservoir, but the higher values of oxred potential are more characteristic for the Riga Reservoir and the Daugava River (Riga), and seasonally higher values were observed also in the Pļaviņas Reservoir. The group of small size individuals (<5 mm) were most strongly and negatively (correlation -0.67) associated with chlorophyll α. Similar to the group of medium size individuals (5-8 mm) were most strongly and negatively associated with pH (correlation -0.62). The group of large size individuals (>8 mm) were very weakly and negatively (correlation -0.10) associated with oxred potential.

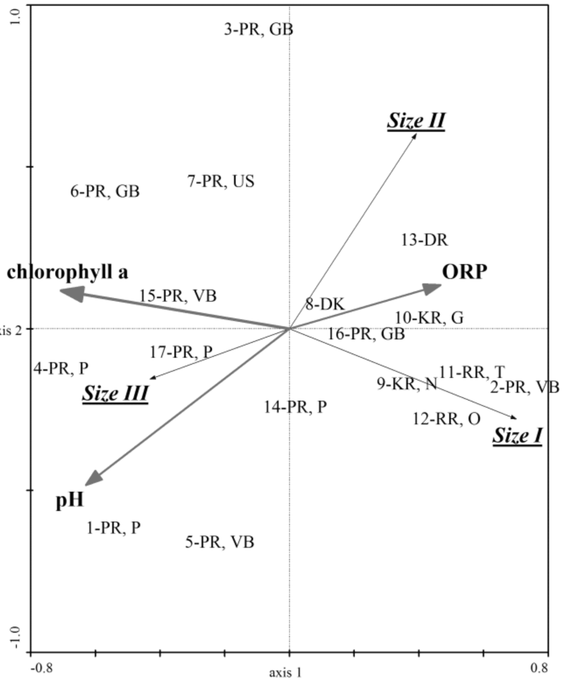


Fig. 5. Ordination diagramm of RDA between data of the individual size groups of *P. robustoides* and environmental variables identified as significant ($p<0.05$) by Monte Carlo permutation test for study area.

PR, P – Pļaviņas Reservoir, Pikstere, PR, VB - Pļaviņas Reservoir, Vārpu backwater, PR, GB - Pļaviņas Reservoir, Gobena backwater, PR, US - Pļaviņas Reservoir, upper stretch, DK – Daugava River, Klidziņa, KR, N – Ķegums Reservoir, Ņega, KR, G – Ķegums Reservoir, Graužupīte, RR, T – Riga Reservoir, Tome, RR, O - Riga Reservoir, Ogre, DR – Daugava River, Riga; ORP – oxred potential

## DISCUSSION

This study shows that in the reservoirs of the Daugava River *P. robustoides* is a dominant amphipod in the Daugava River reservoirs, just as in our previous study (Paidere et al. 2016). But the Ponto-Caspian amphipods *Obesogammarus crassus* and recently detected *Dikerogammarus villosus* in the Riga port (Minchin at al. 2019) are potential invaders in Latvian inland waters. Extinction of indigenous amphipods and change of macroinvertebrate assemblages can occur due to possible invasions of alien amphipods.

*Pontogammarus robustoides* population structure by body length seasonally changed from larger individuals in May to smaller in June and July, the size increased in August and September in the Pļaviņas Reservoir (Fig. 4). Presence of small or juvenile individuals in June, July, and August and in September (ovigerous females were always observed, personal comments) indicated that in the Pļaviņas Reservoir of the Daugava River *P. robustoides* is able to reproduce throughout a season. Research of the Neva Estuary on *P. robustoides* has also demonstrated that the development of three or two generations in year depended on warm or cold weather (Berezina et al. 2017). Similar results about the traits of reproductivity of *P. robustoides* in the Kuibyshev and Saratov reservoirs demonstrate polivoltine life cycle with three generations in a year (Kurina 2016). Further investigations of *P. robustoides* are needed to reveal the role of life-history traits promoting the species invasion in the Daugava River especially compared to indigenous amphipod.

Chlorophyll α and pH were the strongest contributors to the variation in the *P. robustoides* population structure by a body length in the Daugava River. It is evident that small-bodied individuals or juveniles and the medium size individuals are related to different sites within study territory both by water eutrophy and by season. Mainly it is the Riga Reservoir, Ķeguma Reservoir with lowest chlorophyll α and pH values, but the Pļaviņas Reservoir is with higher chlorophyll α and pH values. While the adults or large individuals are weakly related to lower oxred potential in the Pļaviņas Reservoir, seasonally it is May and July. Such division is supported by the other investigation and could be explained by the species feeding behaviour during ontogenetic or size development, by environment conditions, by type of habitats and by season. Detailed studies of the feeding behaviour within different environments (the eutrophic/hypereutrophic Neva Estuary, Curonian Lagoon and the mesotrophic Lake Plateliai) of *P. robustoides* based on stable isotope and gut content analysis showed that *P. robustoides* change from herbivores/detritivores at juvenile stages within both eutrophic and mesotrophic environment to detritivores/omnivores or carnivores within more eutrophic environment at adult stage that depends on quality and quantity of feeding resources. Furthermore, *P. robustoides* is characterized by high stoichiometric plasticity, and this may explain why alien peracaridan species from the high eutrophic freshwaters indicate the substantially higher invader potential comparison with freshwaters of low trophy (Berezina et al. 2017, Arbačiauskas et al. 2013, Bacela-Spychalska, Van der Velde 2013, Berezina 2007). Considering that the Daugava River and its reservoirs are characterised as eutrophic but the limiting nutrient there is phosphorus and following the above-mentioned *P. robustoides* could be characterised as more detritivores/ herbivores and omnivores in the Daugava River and its reservoirs. According to our results, the juveniles of *P. robustoides* are more herbivore (plant detritus, periphyton (Berezina et al. 2009)) especially in the sites with lower eutrophy, and large individuals are more detritivore or omnivore in the sites of the Daugava River and its reservoirs with higher eutrophy. Detritus or particulate organic materials contain both living and dead organic parts. The lower oxred potential could indicate the organic-rich sediments and the presence of other macro-invertebrates (Poznańska et al. 2010). The studies of trophic position and predatory abilities of invasive *P. robustoides* using stable isotope and gut content analysis in Poland water-bodies showed that adult *P. robustoides* took a high trophic position. Detritus and especially animal materials (animal tissue, oligochaetes and other) are the most important food dietary (Bacela-Spychalska, Van der Velde, 2013). Similar results about food spectra in the Neva Estuary and the Curonian Lagoon showed that *P. robustoides* adult individuals are omnivorous, consuming aquatic insect larvae, oligochaetes, isopods, and other crustaceans as well as filamentous algae (Berezina et al. 2017).

One of the important abiotic factors in running waters and reservoirs is hydrological regime, especially water level changes (Lampert, Sommer, 2007). Very shallow depth and shore-near zones by sands, gravels, stones, filamentous algae and macrophytes stands are typical freshwater habitats for *P. robustoides,* but at the same time these habitats are the most exposed to water level changes. It was observed that *P. robustoides* occurred at the submerged sandy bottom sites in the flooded areas in the Włocławek Reservoir in Poland (Poznańska et al. 2010). Our results showed that the medium size individuals of *P. robustoides* prefer habitats with the lowest water and seasonally it coincides with August. It is could be due to the low level of warm stagnant water, food availability and decrease of competition. It is supported by experimental studies about abiotic factors affecting microhabitat selection by alien *P. robustoides*. For example, *P. robustoides* generally prefer warm water and avoided flow ≥15 cm $s^{-1}$. According to studies about variable depth and substrata preferences of *P. robustoides*, the species selected shallower locations, limiting inter-specific competition, allowing optimal utilization of feed and decreasing predatory pressure (Kobak et al. 2017, Kobak et al. 2017a, Jermacz et al. 2015). On the other hand, in our subsequent studies in 2017 in the Pļaviņas Reservoir due to heavy rainfall and comparatively higher water levels (July, August, and September) than in 2016, *P. robustoides* was obtained in May and June in one site out of two, but the rest of the season it was not detected (unpublished data, personal comments).

## CONCLUSIONS

In conclusion, our results show that stronger impact on the alien *P. robustoides* population structure, especially immature individuals is caused by the reservoir eutrophic conditions and water level. Immature individuals could be more herbivore and prefer habitats with lower water level. Adult individuals of *P. robustoides* demonstrate more omnivorous behaviour. In the future, if nutrient levels or trophy in particular phosphorus increase in the Daugava River, higher invasion success is to be expected.

## ACKNOWLEDGEMENTS

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