

The influence of population structure and environmental factors on the hydroxyproline and collagen level in Baltic crustaceans

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The aim of the work is to determine the relationship between the structure of the population of the four Baltic crustaceans and the content of hydroxyproline and collagen, as well as to determine the impact of hydroxyproline and collagen on the life processes (growth and reproduction) of these animals. The essence of the research is to determine the relationship between the level of hydroxyproline and collagen and the size, sex and distribution of *Crangon crangon* (Linnaeus, 1758), *Palaemon elegans* Rathke, 1837, *Palaemon adspersus* Rathke, 1837 and *Saduria entomon* (Linnaeus, 1758), as well as the season, temperature and salinity of water.

The organisms for research were collected in 2008 and 2009 in various regions of the Gulf of Gdańsk (Southern Baltic). *C. crangon* was collected in two depth profiles (Gdynia stations: G1 – 10 m, G2 – 14 m, G3 – 17 m, Sopot stations: S1 – 8 m, S2 – 10 m, S3 – 15 m). *P. elegans* and *P. adspersus* were collected in the Yacht Port in Gdynia (PJG station) from the depth of 0.5 – 1 m. *S. entomon* was collected at a depth of 50 m at the Góra Szwedów station (GS station, located in the open part of the Baltic Sea). Within a given species, individuals were divided into four groups: juveniles, males, non-ovigerous females and ovigerous females. In each of these groups, the organisms were assigned to one-centimeter length classes. The level of hydroxyproline was determined in tissues of *C. crangon*, *P. elegans*, *P. adspersus* and *S. entomon* collected in 2008. Collagen extraction and analysis of its hydroxyproline content were performed in the tissues of *C. crangon* and *S. entomon* collected in 2009 and in *P. elegans* collected in 2008. To determine the amount of hydroxyproline in the tissues and in collagen of the examined crustaceans, the colorimetric method was used. Collagen extraction was performed using pepsin. To determine the structure of this protein and its components, polyacrylamide gel electrophoresis was used.

The results indicates that hydroxyproline is involved in various physiological processes in Baltic invertebrates. The level of hydroxyproline varies between the species, which is related to their belonging to different taxonomic groups, and hence their biology and ecology, lifestyle and population structure. Among the four analyzed species of crustaceans, the largest amount of hydroxyproline in tissues has *S. entomon* ($2.13 \pm 0.38 \mu\text{g}/100 \text{ mg DW}$) and the lowest *P. adspersus* ($1.05 \pm 0.17 \mu\text{g}/100 \text{ mg DW}$). Among the three species of decapods, *C. crangon* possessed the highest level of hydroxyproline. The level of this amino acid in *C. crangon*, *P. elegans* and *S. entomon* was determined separately in males, non-ovigerous females and ovigerous females. It is not possible to determine the impact of size and sex on the level of hydroxyproline in *P. adspersus*, because only non-ovigerous females of one length class were used. The results indicates that in *P. elegans*, the sex of individuals has a significant influence on the level of hydroxyproline ($p < 0.05$). This relationship was found only between males and ovigerous females ($p < 0.05$). There were no significant correlations between the other groups ($p > 0.05$). In *C. crangon* and *S. entomon* a significant relationship was found between the sex of individuals and the level of the amino acid ($p < 0.05$). Males of these two species have higher hydroxyproline contents than non-ovigerous females. However, during reproduction period, the ovigerous females possesses the largest amounts of Pro-OH in their tissues. This shows the significant role of this amino acid in the reproductive processes of *C. crangon* and *S. entomon*. It also indicates the different management of hydroxyproline by individuals of different sexes. In *C. crangon*, *P. elegans* and *S. entomon* the hydroxyproline content was analyzed in different length classes. In *P. elegans*, the level of hydroxyproline is not correlated with the body length of males and females ($p > 0.05$), suggesting that these individuals use hydroxyproline equally for both growth and reproduction. In *C. crangon* and *S. entomon* a significant relationship was found ($p < 0.05$) between the size of individuals and the amount of Pro-OH. This indicates that both the production and consumption of hydroxyproline and collagen in these species are closely related to the processes of growth and aging of organisms. The level of hydroxyproline in *C. crangon*, *P. elegans* and *S. entomon* from the Gulf of Gdańsk changes during the year, depending on the stage of organism development and sexual maturity. Environmental factors such as the water temperature and the availability and quality of the food also influence this. The analyzed species have higher hydroxyproline contents as the water temperature increases. Due to slight differences in the salinity of water between the collection stations, it was assumed that this factor does not significantly affect the level of

hydroxyproline. Within the Gulf of Gdańsk, the impact of the area and depth of occurrence on the hydroxyproline level in *C. crangon* was analyzed. The individuals were collected from two relatively close located depth profiles with similar environmental conditions. This resulted in the lack of correlation between the area, the depth of occurrence and the amount of Pro-OH in *C. crangon*. An important reason is also the large shrimp mobility.

Due to the presence of hydroxyproline in the tissues of *C. crangon*, *P. elegans*, *P. adspersus* and *S. entomon* from the Gulf of Gdansk, it was found that they contain collagen. The highest average content of this protein was observed in *C. crangon*, and the smallest in *S. entomon*. More collagen was obtained from male *C. crangon* and *S. entomon* compared to females. However, the level of hydroxyproline in collagen of *C. crangon* is higher in females than in males, and the highest values were found in ovigerous females. The collagen level is similar in males and females of *P. elegans*. Electrophoretic separation of this protein, indicate differences between the examined species in the molecular mass of the subunits. The composition of subunits in collagen extracted from tissues of crustaceans inhabiting the Gulf of Gdansk is similar to vertebrate collagen-type I.

The results indicates that hydroxyproline is an important amino acid in physiological processes of crustaceans from the Gulf of Gdańsk. It can be an indicator of physiological changes in the individuals, and also in a given population. The relationship between the structure of the population and the level of hydroxyproline has been demonstrated, which is confirmed by changes in the content of this amino acid depending on the size and sex of individuals of a given species at certain times of the year. The obtained results allow to conclude that hydroxyproline plays an important role in the body development and growth, and in reproductive period of the studied species. Additionally, information about Pro-OH content, allowed to confirm the presence of collagen in the tissues of crustaceans from the Gulf of Gdansk and to undertake a more accurate study of this protein. The differences in the level and composition of the collagen subunits between crustaceans from the Gulf of Gdańsk indicate the diversity and complexity of its construction. This may suggest that both collagen and hydroxyproline are used in different way, and it depends on the species and individual characteristics of an animal, as well as the environmental conditions.