Oceanological and Hydrobiological Studies

International Journal of Oceanography and Hydrobiology

Volume 48, Issue 2, June 2019

pages (116-124)

ISSN 1730-413X
eISSN 1897-3191

Crangon crangon: can hydroxyproline be an indicator of changes in the species?

by

Agnieszka Kąkol*, Anna Szaniawska

DOI: **10.1515/ohs-2019-0012**
Category: **Original research paper**
Received: **June 14, 2018**
Accepted: **September 24, 2018**

University of Gdańsk, Faculty of Oceanography and Geography, Institute of Oceanography, Department of Experimental Ecology of Marine Organisms, Al. M. Piłsudskiego 46, 81-378 Gdynia, Poland

* Corresponding author: *kakol.agnieszka.gdynia@gmail.com*

Abstract

The hydroxyproline content in *Crangon crangon* tissues from the Gulf of Gdańsk (southern Baltic) was determined in males, non-ovigerous females and ovigerous females, depending on the individual body length, the study area (two profiles: Gdynia and Sopot) and the depth of occurrence. Individuals were collected and analyzed from April to August 2008. The research on the migratory species *C. crangon* indicates that the area and depth of its occurrence do not significantly affect the level of hydroxyproline in the tissues of this animal ($p > 0.05$). However, certain trends have been observed. Hydroxyproline participates in various life processes of *C. crangon* and its level in the tissues is significantly correlated with the sex of animals ($p < 0.05$). In males, hydroxyproline plays a major role in the body growth. Moreover, water temperature significantly affects the hydroxyproline content in males of different body sizes. Ovigerous females use hydroxyproline in the reproductive process. In non-ovigerous females, hydroxyproline participates both in the growth of organisms and in the reproductive period. Hydroxyproline can be an indicator of the collagen level, as well as an important factor in physiological processes.

Key words: hydroxyproline, *Crangon crangon*, crustaceans, collagen, the Gulf of Gdańsk (southern Baltic)

DE GRUYTER

The *Oceanological and Hydrobiological Studies* is online at www.oandhs.pl

Introduction

Crangon crangon (Linnaeus, 1758) is a common crustacean in European seas (Holthuis 1980; Jeffery & Revill 2002; Oh et al. 1999; Revill & Holst 2004) and has a large and stable population in the Gulf of Gdańsk (Baltic Sea) (Żmudziński & Ostrowski 1990; Łapińska & Szaniawska 2006). The stability of this species in this region allows a number of processes to be followed. Certain regularities can thus be established for this population and extrapolated to other populations from different latitudes. The body of shrimps is rich in highly nutritional substances, e.g. protein, unsaturated fatty acids and mineral compounds (Heu et al. 2003; Revill & Holst 2004; Sachindra et al. 2005; Kurita 2006). Biological activity of protein-containing tissues and organs of marine animals depends on its amino acid composition (Yoshinaka et al. 1989; Sivakumar et al. 1997). It is assumed that the amino acids occurring in aquatic invertebrates are involved in various processes and can affect the body development or the condition of individuals (Hulmes 2008; Kavitha & Thampan 2008). One of them is hydroxyproline, an amino acid typical of collagen (Ignat'eva et al. 2007). Hydroxyproline is a characteristic constituent of collagen, accounting for 13 to 14% (Neuman & Logan 1949) or 12.8 to 14.7% (Ignat'eva et al. 2007) of the amino acids forming this protein. Knowing the hydroxyproline content, we can estimate the amount of collagen in a given material, although we have to be aware of the fact that the method of converting the hydroxyproline level into the quantity of collagen may vary slightly depending on the group of animals we are dealing with (Ignat'eva et al. 2007). Siddiqi et al. (2001) assumed that hydroxyproline constitutes 12.5% of collagen. In the case of the shrimps *Penaeus monodon* and *Penaeus vannamei*, Sriket et al. (2007) used the conversion factor of 11.42 suggested by Sato et al. (1986). Mizuta et al. (1998) estimated the amount of collagen in the Antarctic krill *Euphausa superba* based on the fact that hydroxyproline accounts for 12.7% of that protein. Recent research has shown that hydroxyproline, and hence collagen, is present in different parts of the invertebrate body and participates in a range of life processes (Hulmes 2008; Kavitha & Thampan 2008). For example, collagen is involved in the regeneration of animals and in the modulation of stress during swimming (Féral 1988; Sivakumar & Chandrakasan 1999). Collagen synthesis has been shown to intensify wherever the body has been damaged (Féral 1988). Hydroxyproline significantly affects the temperature of collagen denaturation, which is important in the context of thermal stability of this protein (Muyonga et al. 2004). Water temperature affects the level of hydroxyproline and thus collagen content in animals (Furukawa et al. 1973; Shuster et al. 1975; Kavitha & Thampan 2008). This applies in particular to species exposed to changing ambient temperatures (Brauer et al. 2003). The level of hydroxyproline is higher in organisms living in warm waters (Brauer et al. 2003). A number of studies indicate the influence of the sex of animals, water salinity and dietary composition on hydroxyproline and collagen levels in invertebrates (Sato et al. 1978; Vasanthakumar et al. 1980; Touhata et al. 2000; Brauer et al. 2003).

The objective of this work was to define the level of hydroxyproline in *C. crangon* from the Gulf of Gdańsk (southern Baltic). Knowing the quantity of hydroxyproline, one can determine the level of collagen and explain whether and how this protein participates in various life processes of this shrimp. This research should indicate 1) whether the hydroxyproline content is only associated with the growth and ageing of shrimps, or whether it may also be involved in other processes such as reproduction; 2) whether, in view of the evident sexual dimorphism in *C. crangon*, collagen synthesis is correlated with the sex of animals; 3) whether, despite the stable, uniform population in the Baltic, the hydroxyproline content is determined by the distribution of individual animals; 4) whether changes in the levels of this protein are due to environmental factors such as water temperature and food availability.

Materials and methods

Fieldwork

The material for analysis was collected in 2008 at six sites in the Gulf of Gdańsk (southern Baltic) divided into two profiles – Gdynia and Sopot (Table 1). The animals were captured using a drag net (30 × 60 cm; mesh size 2.5 cm). On each occasion, salinity (S = PSU) and temperature (T = °C) of water were measured using a MULTI 340i probe (WTW, Germany).

Laboratory work

The animals were deep-frozen (T = −80°C) and subsequently stored in this state until required for analysis. After thawing, the total length of animals was measured from the tip of the rostrum to the end of the telson, with an accuracy of 0.1 mm. They were also weighed on an electronic scale (XS 205 Dual Range – Mettler Toledo, Poland), with an accuracy of 0.001 g. The animals were sexed and ovigerous females were included in a separate group. They were then divided into three length classes, i.e. 21.0–30.0 mm, 31.0–40.0

mm and 41.0–50.0 mm. Preparations were made from the tissues as well as from the thoracic and abdominal muscles of each shrimp. In the ovigerous females, the thoracic tissues were separated from the eggs.

The prepared tissues and muscles were placed in a desiccator (KBC-65G; Wamed, Poland) and dried at 105°C to constant mass. The hydroxyproline levels in male and female *Crangon crangon* from the three length classes were measured using Stegemann's technique (1958) modified by Czarnowski & Krechniak (1989). The analysis is based on the spectrophotometric measurement of pink color, which is the effect of the condensation reaction of products resulting from the oxidation of hydroxyproline (Hyp) with p-dimethylaminobenzaldehyde (PABA). Samples of 100 mg (n = 3 replicates) were hydrolyzed with 6N HCl at 120°C for 2.5 h. To determine the actual content of hydroxyproline in the material, 200 µl of hydroxyproline standard (trans-4-hydroxy-L-proline ≥ 99%, Sigma-Aldrich) were added to one of the vials. The product of hydrolysis was neutralized with 6N NaOH to pH = 7.0, and then centrifuged at 2800 rpm. Chloramine T was added to a suitably diluted supernatant in the amount of 1 ml, then 1 ml of $HClO_4$ was added after 20 min, and 1.5 ml of p-dimethylaminobenzaldehyde after another 5 min. The solutions were heated over a water bath at 60°C for 18 min. After the test-tubes were cooled, the absorbance of the solution at a wavelength of 555 nm was measured using a UV–1202 spectrophotometer (SHIMADZU Europe GmbH). Quartz cuvettes with a 10 mm optical path length were used.

Calculation of hydroxyproline

A calibration curve of hydroxyproline (Hyp) was plotted, on the basis of which the hydroxyproline content in a dry mass sample of 100 mg was calculated for each type of material. For this purpose, solutions of hydroxyproline (10 µg ml^{-1}) of known concentration were prepared: 0.1, 0.2, 0.3, 0.4, 0.5 µg ml^{-1} along with a blank test. Three replicates of each concentration were prepared. Knowing the absorbance of the material in each length class, it was possible to read the hydroxyproline concentration per 100 mg from the plot.

$$y = ax + b$$
$$x = (y - b) / a$$

where:
- *a* – gradient
- *b* – intercept
- *y* – absorbance of a sample without the standard
- *x* – Hyp content in a sample without the standard

Statistics

Values were expressed as a mean together with the standard deviation (mean ± SD) for a specified number (n) of length classes. The relationship between the studied parameters was determined using the linear equation $y = ax + b$, at a significance level of 5%. Differences between the groups of results were tested with the Mann-Whitney U-test at a level of 5%.

Table 1

Study area, depth, geographical coordinates, and temperature and salinity of water in the following months of 2008

Sampling sites	Depth	Geographical coordinates	Water temperature					Salinity				
	m	λ, ϕ	°C					PSU				
			IV	V	VI	VII	VIII	IV	V	VI	VII	VIII
Gdynia profile												
G1	10	18°36.4'E 54°27.9'N	6.2	13.6	11.2	18.0	18.0	6.2	6.0	6.6	7.1	7.0
G2	14	18°40.0'E 54°29.0'N	6.1	10.1	11.3	17.9	17.5	6.3	6.7	6.7	7.0	7.0
G3	17	18°41.4'E 54°29.4'N	5.7	8.9	11.5	16.3	16.9	6.5	6.8	6.6	7.0	7.0
Sopot profile												
S1	8	18°36.1'E 54°26.4'N	7.1	14.9	11.2	18.1	18.3	6.0	5.3	6.5	7.0	7.0
S2	10	18°37.9'E 54°27.5'N	6.9	13.8	11.2	17.6	17.6	5.9	5.9	6.5	7.0	7.0
S3	15	18°39.7'E 54°28.6'N	6.5	11.2	11.3	16.9	17.0	6.3	6.5	6.6	7.1	7.0

Results

Fieldwork

Water temperature and salinity

The near-bottom water temperatures in the two profiles (Gdynia and Sopot) differed only slightly and changed throughout the year (Table 1). The lowest mean water temperatures were recorded in April (6.8 ± 0.3°C in Gdynia and 6.3 ± 0.2°C in Sopot), and increased in the following months. The highest temperature at all sites was recorded (18°C) in July and August.

The mean salinity remained at the same level at all sites throughout the study period (6.6 ± 0.4 PSU; Table 1).

Laboratory work

A total of 2886 individuals of *C. crangon* were collected during the whole study period – 1302 males and 1291 females. Of the total number of females, 293 were ovigerous from May to August 2008. The largest numbers of *C. crangon* were recorded at the shallowest sites in both profiles (Table 2). All animals captured were used to determine the hydroxyproline levels.

Sex and size of individuals

The mean hydroxyproline levels in males were 1.81 ± 0.61 μg 100 mg^{-1} d.w. and in non-ovigerous females – 1.71 ± 0.64 μg 100 mg^{-1} d.w. Ovigerous females have significantly higher levels of hydroxyproline in their tissues (2.12 ± 0.65 μg 100 mg^{-1} d.w.) compared to other groups, which applies to both profiles of the study area and all the depths (Fig. 1).

In both the Gdynia and Sopot profiles, the hydroxyproline content increased with the individual body length of males and non-ovigerous females (Fig. 2). Mean values were significantly lower in younger specimens compared to the older ones. In ovigerous females from both profiles, the hydroxyproline levels decreased with the length of individuals (Fig. 2).

Study area

Mean hydroxyproline levels in all the groups of the examined shrimps were similar throughout the study area (Table 3, Fig. 3). The differences were not statistically significant ($p > 0.05$). The average hydroxyproline level did not exceed 2.0 μg 100 mg^{-1} d.w. in both males and non-ovigerous females (Fig. 3). Ovigerous females from the Gdynia profile had on average 15% lower content of hydroxyproline in their tissues compared to individuals collected in the Sopot profile (Table 3).

Depth

Males collected at the shallowest sites in both profiles of the study area (site G1 and S1) had the lowest hydroxyproline levels (Fig. 3). The highest values were determined at the deeper sites: G2 (14 m) and S3 (15 m) (Fig. 3). The mean hydroxyproline levels in non-ovigerous females varied with depth. The lowest values were observed at site G2 (14 m) and S3 (15 m). The highest values were determined at site G3 (17 m) and S1 (8 m) (Fig. 3). The average content of hydroxyproline in ovigerous females was at a similar level at all depths in the Gdynia profile (Fig. 3). There was a slight decrease in hydroxyproline levels with depth in the Sopot profile (Fig. 3).

Table 2

The number of *C. crangon* males, non-ovigerous females and ovigerous females in length classes (mm)

Sampling sites		Number of individuals in length classes (mm)								
		Males			Non-ovigerous females			Ovigerous females		
		21.0–30.0	31.0–40.0	41.0–50.0	21.0–30.0	31.0–40.0	41.0–50.0	21.0–30.0	31.0–40.0	41.0–50.0
Gdynia profile	G1	193	95	1	74	109	28	1	24	3
	G2	117	20	0	84	24	3	6	36	2
	G3	51	15	0	64	52	3	1	25	2
Sopot profile	S1	246	115	0	103	173	19	1	69	20
	S2	201	55	0	125	130	13	3	40	9
	S3	106	24	0	81	88	4	1	45	5
Total number		1302			1291			293		

DE GRUYTER

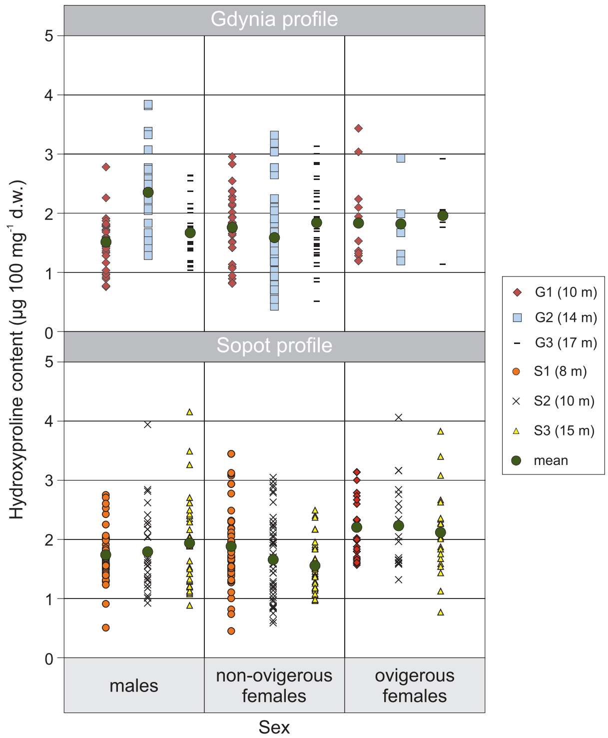

Figure 1

Gdynia and Sopot profiles: hydroxyproline levels (µg 100 mg^{-1} d.w.) in *C. crangon* males, non-ovigerous females and ovigerous females. Mean value from triplicate determinations

Table 3

Hydroxyproline levels in males, non-ovigerous females and ovigerous females at two profiles

Study area	Males	Non-ovigerous females	Ovigerous females
Gdynia profile	1.80 ± 0.64	1.74 ± 0.66	1.88 ± 0.65
Sopot profile	1.83 ± 0.59	1.74 ± 0.63	2.20 ± 0.63

Values are given as means ± SD (Standard Deviation) from triplicate determinations and expressed as µg Hyp × 100 mg^{-1} d.w. (Dry Weight)

Hydroxyproline levels from April to August 2008

Gdynia profile:

A 30% increase in the mean hydroxyproline level was observed in males from April to May (Fig. 4). In the following months, the hydroxyproline content declined. In non-ovigerous females, the lowest value of the analyzed amino acid was observed in May, and the highest one in June (Fig. 4). Ovigerous females appeared in May and then reached the highest mean hydroxyproline levels in their body (Fig. 4). In the following months, the amino acid level decreased.

Sopot profile:

From April to June, mean hydroxyproline levels in males slightly increased and then decreased in subsequent months (Fig. 4). Low mean hydroxyproline

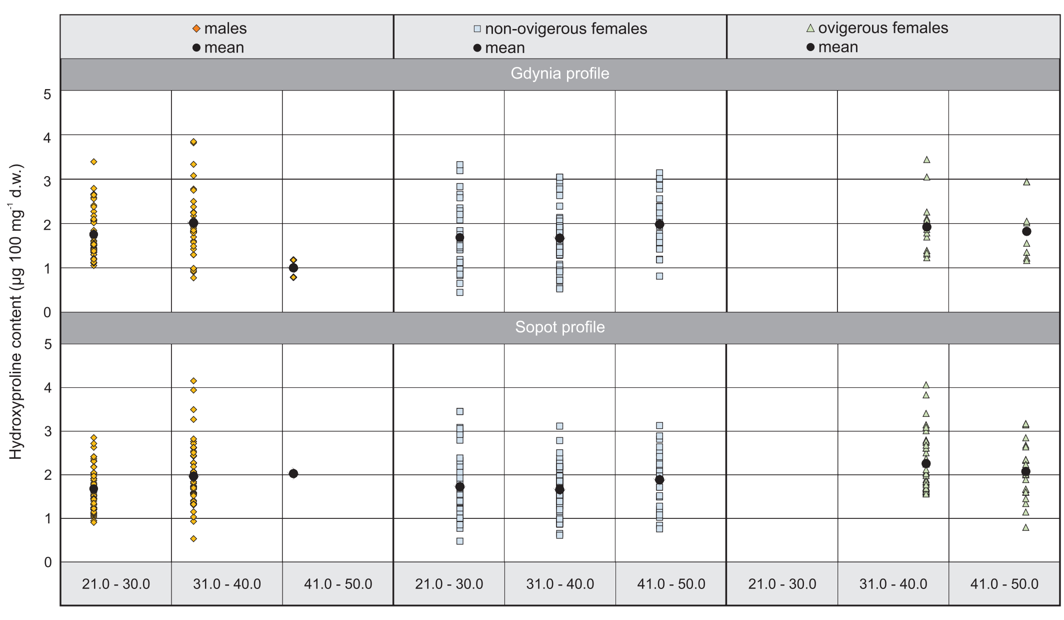

Figure 2

Gdynia and Sopot profiles: hydroxyproline content (µg 100 mg^{-1} d.w.) in *C. crangon* in different length classes. Mean value from triplicate determinations

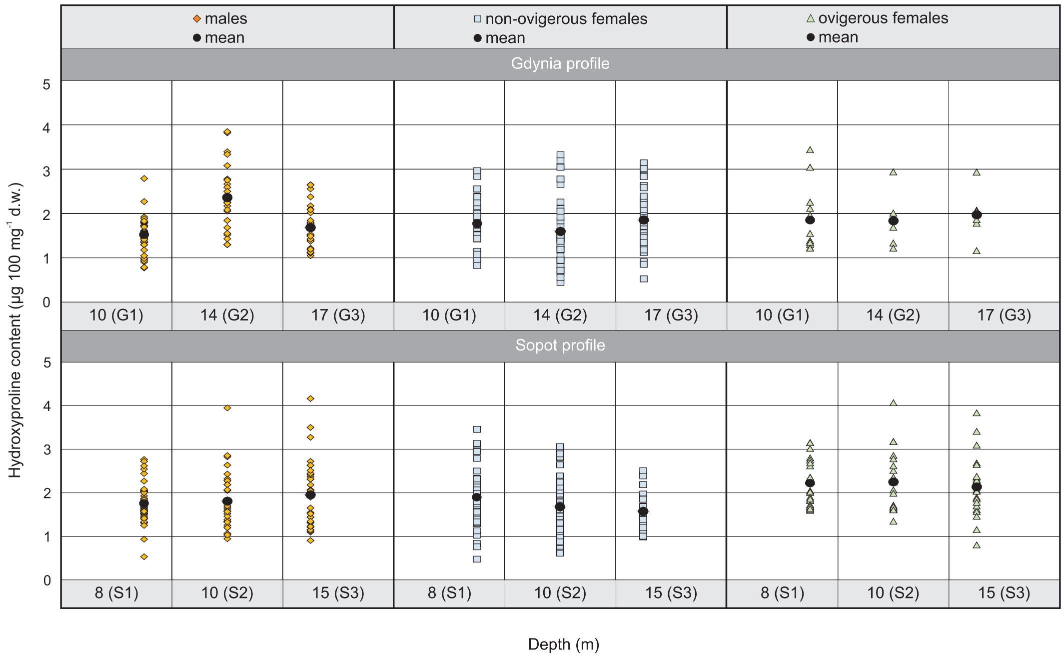

Figure 3

Gdynia and Sopot profiles. Hydroxyproline levels (μg 100 mg^{-1} d.w.) in *C. crangon* males, non-ovigerous females and ovigerous females collected from different depths. Mean value from triplicate determinations

content in non-ovigerous female tissues was observed in May and August, and the highest content was found in July (1.91 ± 0.56 μg 100 mg^{-1} d.w.). In ovigerous females, the mean hydroxyproline level increased by 34% from May to June and then decreased to 1.78 ± 0.17 μg 100 mg^{-1} d.w. in August (Fig. 4).

Discussion

The level of hydroxyproline was determined in the shrimp *C. crangon* from the coastal waters of the Gulf of Gdańsk, where the species has established a permanent population (Żmudziński & Ostrowski 1990; Łapińska & Szaniawska 2006). Our studies show that *C. crangon* uses this amino acid in various life processes. The development and growth of individuals are significantly related to the chemical composition of a protein that builds their tissues. The age and size of an organism is a factor strongly correlated with the hydroxyproline level in tissues. Usually, the rate of collagen synthesis in cells is inversely proportional to age and is associated with growth hormones and the presence of ascorbic acid, which in turn has a significant effect on the enzyme catalyzing the production of hydroxyproline (Touhata et al. 2000; Kavitha & Thampan 2008). The hydroxyproline content in males and non-ovigerous females of *C. crangon* from the Gulf of Gdańsk was found to increase with body length. Changes in the hydroxyproline level in the length class of 21 to 40 mm of these shrimps likely result mainly from collagen synthesis during their growth. On the other hand, the clear decrease in the hydroxyproline content in males from the largest length class (41.0–50.0 mm) is probably the effect of senescence. Morales et al. (2000) observed a higher level of collagen in adult compared to immature cephalopods *Todaropsis eblanae*, which could indicate intensive collagen production during sexual maturation, growth and development of the animal. Muyonga et al. (2004) also recorded higher hydroxyproline levels in adult compared to immature Nile perch (*Lates niloticus*). Akel (1981) observed an increase in the level of collagen formed with age and size of the shrimp *Pandalus jordani*, however, it contained very limited amounts of hydroxyproline. In the present work, it was found that hydroxyproline levels in ovigerous females of *C. crangon* decreased with body length. It has to be emphasized, however, that ovigerous females were not present in the length classes smaller than 31.0–40.0 mm, since they do not become sexually mature until they reach the age of two, and the length of 30–55 mm (Lloyd & Yonge 1947; Tiews 1970; Oh & Hartnoll 2004).

It was once believed that changes in the content of hydroxyproline, and hence of collagen in aquatic

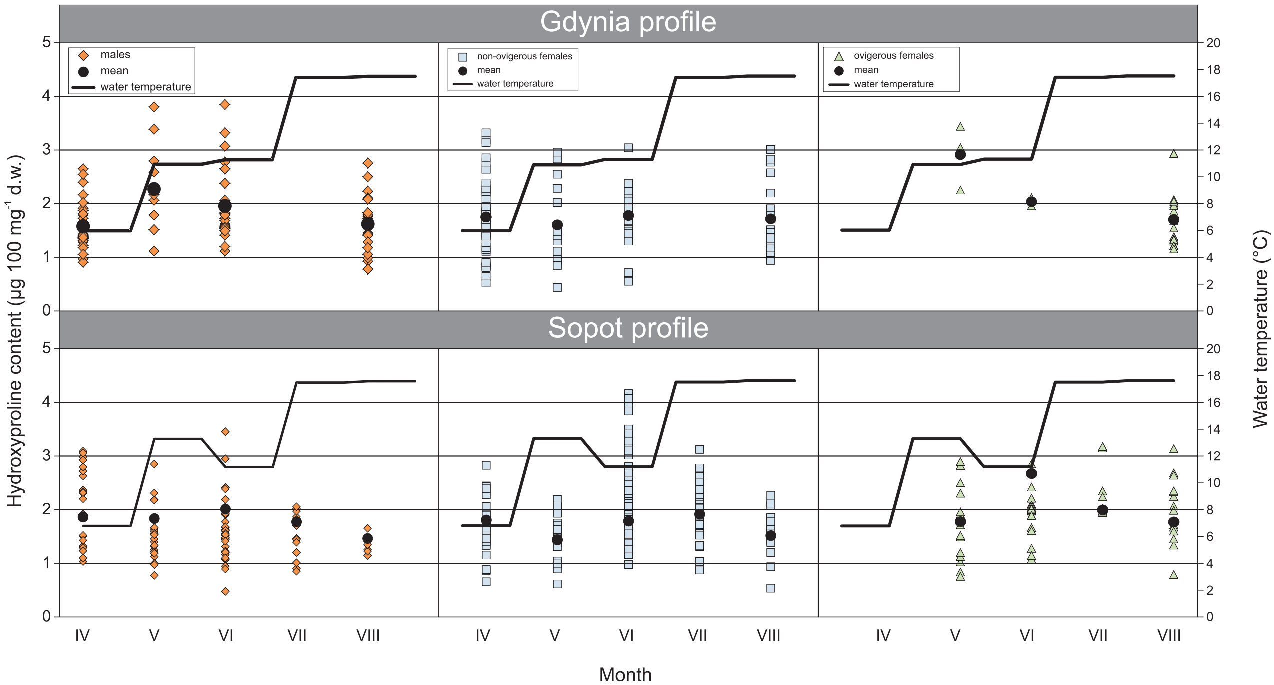

Figure 4

Gdynia and Sopot profiles. Hydroxyproline levels (µg 100 mg^{-1} d.w.) in *C. crangon* males, non-ovigerous females and ovigerous females collected from April to August 2008. Mean value from triplicate determinations

invertebrates, were primarily associated with the age and size of animals (Kavitha & Thampan 2008). It appears, however, that collagen probably participates in a variety of processes, including maturation and egg production. Morales et al. (2000) demonstrated a significant effect of the sex on collagen levels in the cephalopods *T. eblanae* and *Eledone cirrhosa*, in which these levels were higher in females than in males. Touhata et al. (2000) pointed out that during the reproductive period, sex hormones increase the rate of collagen synthesis in the body. In the case of *C. crangon* from the Gulf of Gdańsk, this may indicate a much higher level of hydroxyproline in ovigerous females than in males and non-ovigerous females. In some fish species, collagen levels decrease in both males and females during the reproductive period, although in this context it is very important to know whether the fish die after spawning (Toyohara et al. 1997).

Since the population of *C. crangon* in the Gulf of Gdańsk is homogeneous, notwithstanding sexual dimorphism and differences resulting from reproductive processes, this region has no significant effect on hydroxyproline levels. However, there is a correlation between the hydroxyproline content and the depth at which the shrimps live. Hydroxyproline levels, both in males and non-ovigerous females, increased with depth in the Gdynia profile. In the Sopot profile, the hydroxyproline content in males increased with depth, and in non-ovigerous females – decreased with depth. Presumably, the food conditions affected these amino acid levels to a greater extent than water temperature.

The hydroxyproline content in *C. crangon* from the Gulf of Gdańsk depends on the season, as these shrimps migrate between the coastal zone and the deeper waters of the Gulf. These movements are governed by the availability of food, temperature and oxygenation of water, as well as the reproductive period (Łapińska & Szaniawska 2006). Water temperature is often a factor responsible for changes in a population and affects the life cycles of organisms. In aquatic organisms, a high level of hydroxyproline is directly responsible for the lower susceptibility of collagen to denaturization (Brauer et al. 2003). In both males and females of *C. crangon* from the Gulf of Gdańsk, high levels of hydroxyproline were found at the beginning of the season, in April and May. The lowest water temperature was observed in April 2008. This is the time when shrimps prepare for reproduction, while the abundant food available promotes individual development in the population, increases in the body size and the storage of substances in the tissues to be used in the coming months. The dietary composition of these animals may be of great importance. Research by Sato et al. (1978)

has shown that a diet poor in ascorbic acid leads to a decrease in the level of hydroxyproline, and hence, of collagen. Ovigerous females do not appear before May, but then they have the highest concentrations of hydroxyproline in their tissues. This significantly affects the total amount of hydroxyproline in this species at that time. It decreases at the end of the season in August, which does not correspond with the measured water temperature – the highest in the entire study period.

It may be concluded that hydroxyproline and thus collagen participates in different life processes of *C. crangon*. Male individuals use this amino acid mainly in the body growth, whereas females – both in the body growth and the reproductive period. Our studies show that the hydroxyproline level in *C. crangon* is correlated with the sex and the size of individuals. The hydroxyproline content increased with body length in both males and females (except for ovigerous females). Moreover, the hydroxyproline levels in *C. crangon* are seasonally dependent. The highest hydroxyproline levels are found in spring, due to the maturation processes in specimens and food availability. Despite numerous reports in the world literature on the presence and properties of hydroxyproline and thus collagen in invertebrates, there are no reports on the amino acid content of collagen in Baltic invertebrates, especially representatives of the macrozoobenthos. These are the first studies that determine the level of hydroxyproline in *C. crangon* from the southern Baltic, depending on biotic and abiotic factors.

Acknowledgements

This work was supported by the University of Gdańsk (grant number G 220-5-0242-9).

References

Akel, P.J. (1981). *Characterization of the collagen protein in smooth pink shrimp* (*Pandalus jordani*). Master Thesis (pp. 68), Oregon State University, United States.

Brauer, J.M.E., Leyva, J.A.S., Alvarado, L.B. & Sández, O.R. (2003) Effect of dietary protein on muscle collagen, collagenase and shear force of farmed white shrimp (*Litopenaeus vannamei*). *Eur. Food Res. Tech.* 217: 277–280. DOI: 10.1007/s00217-003-0739-7.

Czarnowski, W. & Krechniak, J. (1989). Hydroxyproline and urinary fluoride in rats repeatedly exposed to inhaled phosphorites. *Fluoride* 22(1): 24–28.

Féral, J.-P. (1988). Wound healing after arm amputation in *Sepia officinalis* (Cephalopoda: Sepioidea). *J. Invert. Pathol.* 52: 380–388. DOI: 10.1016/0022-2011(88)90049-3.

Furukawa, Y., Honma, Y. & Kimura, S. (1973). Age-dependent changes on insoluble collagen of the skin of rats. *Tohoku J. Agri. Res.* 23(4): 216–223.

Heu, M.S., Kim, J.S. & Shahidi, F. (2003). Components and nutritional quality of shrimp processing by-products. *Food Chem.* 82: 235–-242. DOI: 10.1016/S0308-8146(02)00519-8.

Holthuis, L.B. (1980) FAO Species catalogue. vol. 1. Shrimps and prawns of the world. An annotated catalogue of species of interest to fisheries, The Food and Agriculture Organization of the United Nations. Rome. pp. 271.

Hulmes, D.J.S. (2008). Collagen diversity, synthesis and assembly. In P. Fratzl (Ed.), Collagen. Structure and Mechanics (pp. 15–41), New York: Springer.

Ignat'eva, N.Yu., Danilov, N.A., Averkiev, A.V., Obrezkova, M.V., Lunin, V.V. et al. (2007). Determination of hydroxyproline in tissues and the evaluation of the collagen content of the tissues. *J. Anal. Chem.* 62(1): 51–57.

Jeffery, S. & Revill, A. (2002). The vertical distribution of southern North Sea *Crangon crangon* (brown shrimp) in relation to towed fishing gears as influenced by water temperature. *Fish. Res.* 55: 319–323. DOI: 10.1016/S0165-7836(01)00297-1.

Kavitha, O. & Thampan, R.V. (2008). Factors influencing collagen biosynthesis. *J. Cell. Biochem.* 104: 1150–1160. DOI: 10.1002/jcb.21728.

Kurita, K. (2006). Chitin and chitosan: Functional biopolymers from marine crustaceans. *Mar. Biotechnol.* 8: 203–226. DOI: 10.1007/s10126-005-0097-5.

Lloyd, A.J. & Yonge, C.M. (1947). The biology of *Crangon vulgaris* (Linneaus, 1758) in the Bristol Channel and Severn estuary. *J. Mar. Biol. Ass. U.K.* 26: 626–661.

Łapińska, E. & Szaniawska, A. (2006). Environmental preferences of *Crangon crangon* (Linnaeus, 1758), *Palaemon adspersus* Rathke, 1837, and *Palaemon elegans* Rathke, 1837 in the littoral zone of the Gulf of Gdańsk. *Crustaceana.* 79(6): 649–662. DOI: 10.1163/156854006778026799.

Mizuta, S., Miyagi, T. & Yoshinaka, R. (1998). Characterization of a major alpha component of collagen from muscle of Antarctic krill *Euphausa superba*. *Comp. Biochem. Physiol.* 120B: 597–604.

Morales, J., Montero, P. & Moral, A. (2000). Isolation and partial characterization of two types of muscle collagen in some cephalopods. *J. Agri. Food Chem.* 48: 2142–2148. DOI: 10.1021/jf990711k.

Muyonga, J.H., Cole, C.G.B. & Duodu, K.G. (2004). Characterisation of acid soluble collagen from skins of young and adult Nile perch (*Lates niloticus*). *Food Chem.* 85: 81–89. DOI: 10.1016/j.foodchem.2003.06.006.

Neuman, R.E. & Logan, M.A. (1949). The determination of hydroxyproline. *J. Biol. Chem.* 299–306.

Oh, C.-W. & Hartnoll, R.G. (2004). Reproductive biology of the common shrimp *Crangon crangon* (Decapoda: Crangonidae) in the central Irish Sea. *Mar. Biol.* 144: 303–

316. DOI: 10.1007/s00227-003-1205-6.

Oh, C.W., Hartnoll, R.G. & Nash, R.D.M. (1999). Population dynamics of the common shrimp, *Crangon crangon* (L.) in Port Erin Bay, Isle of Man, Irish Sea. *ICES J. Mar. Sci.* 56: 718–733. DOI:10.1006/jmsc.1999.0501.

Revill, A.S. & Holst, R. (2004). Reducing discards of North Sea brown shrimp (*C. crangon*) by trawl modification. *Fish. Res.* 68: 113–122. DOI: 10.1016/j.fishres.2004.02.001.

Sachindra, N.M., Bhaskar, N. & Maheendrakar, N.S. (2005). Carotenoids in different body components of indian shrimps. *J. Sci. Food Agri.* 85: 167–172.

Sato, M., Yoshinaka, R. & Ikeda, S. (1978). Dietary ascorbic acid requirement of rainbow trout for growth and collagen formation. *Nippon Suisan Gakkaishi* 44: 1029–1035. DOI: 10.2331/suisan.58.671.

Siddiqi, N.J., Sharma, B. & Alhomida, A.S. (2001). A study on distribution of different hydroxyproline fractions in the bovine ocular tissues. *Mol. Cell. Biochem.* 217: 67–71. DOI: 10.1023/A:1007236301956.

Sivakumar, P. & Chandrakasan, G. (1999). Marine invertebrate collagens: The prevalence of type V and XI like collagen in some marine crustacean and molluscan tissues. *Proc. Indian Acad. Sci. (Chem. Sci.)* 111(1): 87–104.

Sivakumar, P., Suguna L. & Chandrakasan, G. (1997). Purification and partial characterization of a type V like collagen from the muscle of marine prawn *Penaeus indicus*. *J. Biosci.* 22(2): 131–141. DOI: 10.1007/BF02704727.

Shuster, S., Black, M.M. & McVitie, E. (1975). The influence of age and sex on skin thickness, skin collagen and density. *Brit. J. Dermatol.* 93: 639–643. DOI: 10.1111/j.1365-2133.1975.tb05113.x.

Sriket, P., Benjakul, S., Visessanguan, W. & Kijroongrojana, K. (2007). Comparative studies on chemical composition and thermal properties of black tiger shrimp (*Penaeus monodon*) and white shrimp (*Penaeus vannamei*) meats. *Food Chem.* 103: 1199–1207. DOI: 10.1016/j.foodchem.2006.10.039.

Stegemann, H. (1958). Microdetermination of hydroxyproline with chloramine-T and p-dimethylaminobenzaldehyde. *Hoppe Seylers Z. Physiol. Chem*. 311: 41–45.

Tiews, K. (1970). Synopsis of biological data on the common shrimp *Crangon crangon* (Linneaus, 1758). *FAO Fish. Rep.* 57(4): 1167–1224.

Touhata, K., Tanaka, M., Toyohara, H., Tanaka, H. & Sakagichi, M. (2000). Seasonal change in collagen content of red seabream muscle. *Fish. Sci.* 66: 553–557.

Toyohara, H., Ito, K., Touhata, K., Kinoshita, M., Kubota, S. et al (1997). Effect of maturation on the contents of free and bound forms of hydroxyproline in ayu muscle. *Fish. Sci.* 63(9): 843–844.

Vasanthakumar, G., Chandrakasan, G. & Krishnan, G. (1980). A comparative study of the composition of the collagens of the foot of two lamellibranch molluscs occurring in different habitats. *J. Biosci.* 2(4): 299–304.

Yoshinaka, R., Sato, K., Itoh, Y., Nakajima, S. & Sato, M. (1989) Content and partial characterization of collagen in crustacean muscle. *Comp. Biochem. Physiol.* 94B: 219–223. DOI: 10.1016/0305-0491(89)90037-0.

Żmudziński, L. & Ostrowski, J. (1990). Zoobenthos. In A. Majewski. (Ed.), *Gdańsk Bay* (pp. 402–430), Warszawa: Wyd. Geol. (In Polish).