

# Fatty Acid Composition of Selected Fresh Water Gammarids (*Amphipoda*, *Crustacea*): A Potentially Innovative Source of Omega-3 LC PUFA

Wojciech Kolanowski · Andrzej Stolyhwo ·  
Michał Grabowski

Received: 26 October 2006 / Revised: 19 July 2007 / Accepted: 19 July 2007 / Published online: 11 August 2007  
© AOCS 2007

**Abstract** The aim of this work was to estimate the content and composition of n-3 long chain polyunsaturated fatty acids in selected gammarid species commonly occurring in fresh waters of Central and Western Europe. Five gammarid species were investigated, two native, one old invader consider as semi-native and two Ponto-Caspian species invading the area in the last decade. Lipid content of evaluated species ranged from 75 to 130 g kg<sup>-1</sup> of dry weight. Significant amounts of n-3 LC PUFA were found in the lipid fraction of analyzed gammarids 11–23% of total fatty acids, especially in the Ponto-Caspian species. The main n-3 fatty acid was EPA—up to 16% of total fatty acids. The ratio of n-6 to n-3 was nutritionally desirable, especially in the case of the Ponto-Caspian species. The results obtained and growing biomass of gammarids observed in fresh waters of Central and Western Europe, as well as the rapid depletion of sea fish communities leads us to the idea that these gammarids might be considered as a innovative source of n-3 LC PUFA for nutritional, pharmaceutical and animal feeding purposes.

**Keywords** Amphipods · Crustaceans ·  
Fatty acids composition · Gammarids · Omega-3 PUFA

## Introduction

During last decades it has been well established that long chain omega-3 polyunsaturated fatty acids (n-3 LC PUFA) have a beneficial influence on human health [1]. These fatty acids consist mainly of eicosapentaenoic acid (EPA C20:5), docosahexaenoic acid (DHA C22:6) and, to a lesser extent, docosapentaenoic acid (DPA C22:5). An increased interest in the potential health benefits of n-3 LC PUFA started in the 1970s. This was initiated by epidemiological findings of Bang et al. [2], who discovered that Greenland Eskimos have an extremely low incidence of cardiovascular diseases, despite their diet rich in fat, saturated fatty acids and cholesterol in comparison to continental Danes. The explanation of this phenomenon was high dietary intake of long chain n-3 PUFA of marine origin. This finding stimulated worldwide research resulting in a large number of epidemiological, animal and clinical studies investigating the potentially beneficial health influence of n-3 PUFA, especially long chain EPA and DHA intake [2, 3]. The combined results of these studies suggest that n-3 fatty acids may be a potent factor in the prevention and treatment of many diseases, i.e., cardiovascular diseases, certain types of cancer and diseases with an immuno-inflammatory component [4, 5]. Additionally it was discovered that they are necessary for proper development and functioning of the brain, retina and testis. LC PUFA play a crucial role in cell membranes structure and function [1]. From n-3 LC PUFA many regulatory substances are formed, e.g. specific prostaglandins, prostacyclins, thromboxane and leucotrienes exerting a positive influence on human body functions [5]. The level of n-3 LC PUFA in the body strictly depends on its level in the diet. However, the average level of n-3 LC PUFA intake with the Western-style diet is far below the recommended minimum, which is considered to be 0.2–0.4 g per person per day

W. Kolanowski (✉) · A. Stolyhwo  
Faculty of Human Nutrition and Consumer Sciences,  
Department of Analysis and Quality Assessment of Food,  
Warsaw Agricultural University, SGGW,  
ul. Nowoursynowska 166, 02-787 Warsaw, Poland  
e-mail: wojciech\_kolanowski@sggw.pl

M. Grabowski  
Department of Invertebrate Zoology and Hydrobiology,  
Faculty of Biology and Environment Protection,  
University of Lodz, Lodz, Poland

[6–10]. Moreover, the ratio of n-6 to n-3 PUFA in the diet is recommended to be not higher than 4:1 however, usually it is approximately 10:1 or even higher mainly due to high vegetable fat consumption and low intake of fish [8].

The main source of n-3 LC PUFA for nutritional and pharmaceutical purposes is fish, especially sea fish, as well as other marine animals (e.g. seals, krill) or algae and its oils [11]. Fish cannot synthesize n-3 LC PUFA and its level in the fish body and in fish oil depends on its diet. For sea fish and other marine animals, the main source of n-3 LC PUFA is plankton containing some species of algae and simple fungi synthesizing these fatty acids [12]. Fish incorporate n-3 LC PUFA into body tissues from plankton and predators from plankton-eating fish. Fresh water fish are known to contain a much lower amount of n-3 LC PUFA than those from the sea. However, also some freshwater predatory fish species may also contain high levels of these fatty acids, e.g. trout [13]. The answer to the question of how n-3 LC PUFA are incorporated into trout body tissues may be its feeding behavior and preferences. It is well known among fly-anglers that in natural conditions trout preferably choose gammarids (Amphipoda)—small crustaceans occurring commonly in fresh waters, as well as in seas both cold and warm [14, 15]. Moreover, some biological similarity between gammarids and Antarctic krill (*Euphausia*) suggests that the lipid composition of gammarids may be similar to the composition of krill [15, 16]. This leads to the supposition that the main dietary source of n-3 LC PUFA for trout might be gammarids.

In the last two decades, gradual and permanent increase of gammarid biomass in fresh waters of Central and Western Europe have been observed. This is mainly caused by several species of so-called invasive gammarids species migrating from the basins of Caspian and Black Seas along the river and artificial canal systems to the Central and Western Europe [17]. Examples of such migration are *Pontogammarus robustoides* and *Dikerogammarus haemobaphes*—formally named Ponto-Caspian species [18, 19]. These gammarid species show much higher fecundity, brood size, number of generations per year, salinity tolerance and anthropopression resistance (water pollution, sewages, agrochemicals) than species of native fauna of this region [17, 18, 20]. It was found that gammarid density in fresh and brackish waters of Central and Western Europe (e.g. Odra or Vistula Rivers) increases each year and may reach up to or even exceed  $50 \times 10^3$  animals per  $m^{-2}$ , depending on place, depth and season [21–23]. This leads to an additional supposition that gammarids (of this high biomass increase) might be potentially commercially farmed and used as a innovative source of long chain omega-3 PUFA, as well as a natural feed for farmed trout, as has been done in the case of krill and farmed salmon [24]. This could increase the amount of

nutritionally desirable n-3 LC PUFA in tissues of trout (and other animals) potentially farmed on gammarids as natural and organic aquaculture feed.

Hence the aim of this study was to estimate the content and composition of n-3 LC PUFA in gammarid species most commonly occurring in fresh waters of Central and Western Europe.

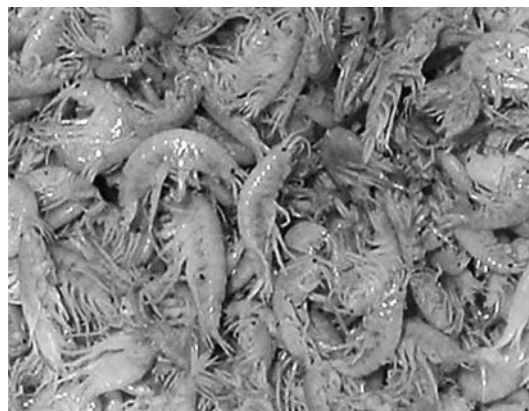
## Experimental Procedures

### Materials

Five gammarid species most commonly occurring in fresh waters of Poland were investigated: *Gammarus fossarum* collected from Struga Dobieszkowska River E19°32', N51°24'; *Gammarus pulex*—from Stradanka River E19°32', N54°18'; *Gammarus roeseli*—from Notec River E18°30', N52°24'; *Pontogammarus robustoides* and *Dikerogammarus haemobaphes*—from Wloclawski Reservoir (built on the Vistula River) E19°30', N52°35'. *G. fossarum* and *G. pulex* were native species typical for benthic fauna of Polish fresh waters. *Pontogammarus robustoides* and *D. haemobaphes* are Ponto-Caspian species which invaded this region in the last decade. *Gammarus roeseli* might be considered as semi-native, this species immigrated into Central Europe many years ago from the Balkans and became a member of the typical fauna of this region. Sampling was carried out in May 2005 (Fig. 1). Additionally wild rainbow trout caught in May 2005 in the Skotawa River E17°15', N54°20' were analyzed.

### Sample Preparation

Freshly caught (live) gammarid samples were quickly taken to our laboratory. Samples were rinsed with fresh



**Fig. 1** Image of analyzed gammarids, example of *Pontogammarus robustoides*

water to eliminate foreign materials. Then, external water was eliminated from the samples by drying on a paper towel under air flow. Samples were measured, weighed and moisture content examined gravimetrically after drying at 70 °C until constant weight.

The lipid fraction was extracted according to the Folch method [25]. One gram of fresh sample was homogenized in a cold mixture of chloroform–methanol (2:1). After filtering the solid residue was washed with a chloroform–methanol mixture and filtered. The combined filtrates were transferred to the measuring cylinder and one quarter of the total volume of the filtrate of water was added. The mixture was shaken thoroughly and the two phases were allowed to separate. The lower chloroform phase was removed, dried over anhydrous  $\text{Na}_2\text{SO}_4$ , then roto-evaporated under vacuum at 40 °C. The lipid fraction obtained was covered by nitrogen, weighed and washed out from the evaporation flask using hexane, additionally dried by passing through anhydrous  $\text{Na}_2\text{SO}_4$ , then closed in a small vials under nitrogen.

To convert fatty acids to methyl esters (FAME), hexane was evaporated by nitrogen flow. The dry lipid fraction was saponified by 0.5 N solution NaOH with methanol, covered with nitrogen, mixed and heated in the water-bath at boiling point for 40 min. The saponified sample was transmethylated with 14%  $\text{BF}_3$  in methanol reagent, covered with nitrogen, at boiling point for 3 min. After that, the mixture was cooled and 3 mL hexane was added, covered with nitrogen and shaken vigorously for 30 s while still warm. Then 40 mL of saturated water solution of NaCl was added and shaken vigorously. After separation, the hexane layer was transferred by syringe to the thin glass tube and additionally dried over anhydrous  $\text{Na}_2\text{SO}_4$  and decanted to clean vial, covered with nitrogen and capped. One  $\mu\text{L}$  of prepared FAME was injected in to the chromatograph under appropriate conditions. As internal standard tricosanoic acid (C23:0) methyl ester was used (Sigma-Aldrich, Steinheim, Germany). FAME were prepared according to slightly modified AOCS method Ce 1b-89 [26].

### Chromatography

The analysis of FAME was performed by GC using an Agilent 6890 N instrument (Agilent, Böblingen, Germany) equipped with Rtx 2330 silica capillary column of 100 m length, 0.25 mm ID,  $d_f$  0.1  $\mu\text{m}$  (Restek Corp, Bellefonte, USA). Hydrogen was used as the carrier gas at a flow rate of 0.9  $\text{mL s}^{-1}$ . A split-splitless (50:1) injector at 235 °C and flame-ionization detector (FID) at 250 °C were used. The column temperature was programmed as follows: initial 155 °C, time 55 min, then increased at 1.5 °C  $\text{min}^{-1}$  to a

final temperature of 210 °C. Each sample was analyzed in triplicate. Results were collected in Chem-station and transformed using software HP-Chem (Hewlett Packard, Palo Alto, USA). Peaks were identified by comparison with known standards: menhaden reference oil (Supelco, Germany) and FAME Mix Supelco 37 (Supelco, Germany). Results were reported as peaks area percentages.

### Data Analysis

The results obtained were statistically analyzed using a one-way analysis of variance (ANOVA) to check the significance of differences in levels of particular fatty acids among measurements and among analyzed gammarid species. Results were analyzed using Statgraphics Plus ver 4.1 software, at a significance level of  $p < 0.01$ .

### Results and Discussion

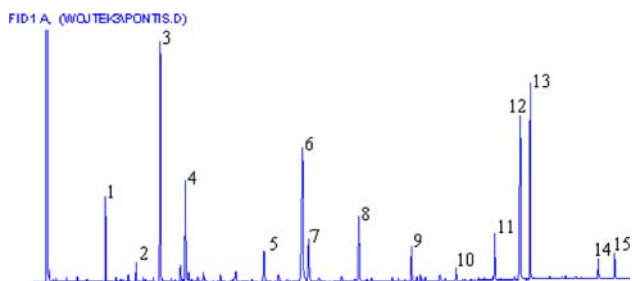
The preliminary evaluation showed differentiation between analyzed gammarid species in mean size, dry weight and lipid fraction. The results are presented in Table 1. The samples of the Ponto-Caspian species were significantly greater in size and weight than the native ones. The highest amount of the lipid fraction was shown by *P. robustoides*, the lowest one in the case of native *G. pulex*. In the lipid fraction of analyzed gammarids 43 fatty acids were found. However, significant levels were shown for 15 fatty acids (Fig. 2; Table 2).

The predominant fatty acids in all gammarids samples was monounsaturated C18:1 n-9 (19–23%) and saturated C16:0 (17–24.5%). The other fatty acids of comparatively high level were polyunsaturated C18:2 n-6 linoleic acid (5.1–18%) and C20:5 n-3 EPA (5–16%) and monounsaturated C16:1 (5.5–10%). Differentiation in the level of n-3 LC PUFA among the samples was observed, ranging from 11.1 to 31.1% of total fatty acids (Fig. 3). The highest level of n-3 LC PUFA was found in *D. haemobaphes* and *P. robustoides*, which did not differ significantly from each other. Percentage ranges of particular n-3 LC PUFA were: EPA 5.1–16.2, DPA 0.5–2.4, DHA 1.5–4.2 of all fatty acids. The total level of omega-3 LC PUFA in wet weight averaged 1.4–7.8  $\text{g kg}^{-1}$ , i.e., 5.3–28.3  $\text{g kg}^{-1}$  in dry weight, depending on the gammarid species (Fig. 4). The highest level of n-3 LC PUFA was found in *P. robustoides*, the lowest in *G. pulex*. The ratio of n-6 to n-3 PUFA was significantly lower (two or three times) in the lipid fraction of Ponto-Caspian species than in the native ones (approximately 1:2.5 and 1:0.8, respectively).

Comparison of analyzed gammarid species to trout showed that the fatty acids composition of the gammarids

**Table 1** Mean basic composition of analyzed gammarid samples

Gammarid species	Mean weight (mg)	Weight range (mg)	Mean size (cm)	Size range (cm)	Dry weight, mean (g kg <sup>-1</sup> )	Lipids in wet weight (g kg <sup>-1</sup> )	Lipids in dry weight (g kg <sup>-1</sup> )
<i>G. fossarum</i>	9.7	4.8–14.6	0.8	0.5–1.5	268.2	29.5	109.9
<i>G. pulex</i>	8.6	4.7–12.5	0.7	0.4–1.3	269.3	20.0	75.1
<i>G. roeseli</i>	20.1	10.9–29.3	0.9	0.6–1.6	266.0	20.1	77.0
<i>D. haemobaphes</i>	56.2	42.2–70.3	1.8	1.2–2.0	270.1	21.3	78.9
<i>P. robustoides</i>	62.4	47.8–76.7	2.1	1.5–2.4	275.3	36.1	131.1



**Fig. 2** Chromatogram of FAME analysis, example of *Pontogammarus robustoides*, 1 C14:0, 2 C15:0, 3 C16:0, 4 C16:1, 5 C18:0, 6 C18:1n-9, 7 C18:1n-7, 8 C18:2n-6, 9 C18:3n-3, 10 C20:1, 11 C20:4n-6, 12 C23:0 internal standard, 13 C20:5n-3 EPA, 14 C22:5n-3 DPA, 15 C22:6n-3 DHA

was similar to trout in the total content of saturated, mono and polyunsaturated fatty acids. However, the profile of polyunsaturated fatty acids differed significantly. The main difference was found in the levels of EPA and DHA. In all analyzed gammarids the predominant n-3 LC PUFA was EPA, DHA occurred in much less extent (5–16.2% and 1.5–4.2%, respectively). In contrast, trout (as well as other fish) contained significantly lower levels of EPA than DHA. The ratio of n-6 to n-3 PUFA in the lipid fraction of Ponto-Caspian species was similar to the one of trout and significantly lower than in the native and semi native species (Table 2).

Differences in PUFA composition were also found among gammarid species. Native *G. fossarum* and *G. pulex*, as well as semi-native *G. roeseli* contained much higher amounts of linoleic acid C18:2 n-6 (13.0–18.2%) than the Ponto-Caspian species (5.1–6.2%). Similarly in the case of alpha-linolenic acid C18:3 n-3, the natives contained its higher amount than the Ponto-Caspian species (4.5–7.1% vs. 2.2–2.5%, respectively). In the case of long chain PUFA the EPA level was found to be significantly higher in the Ponto-Caspian species than in the native ones (16% vs. 5–9%, respectively), in *G. roeseli* the level of EPA was intermediate (13%). Less differentiation but, however, also significant, was observed in the DPA and DHA levels. In general, both DPA and DHA levels were significantly higher in the Ponto-Caspian species (1.6–2.4% and 3.3%,

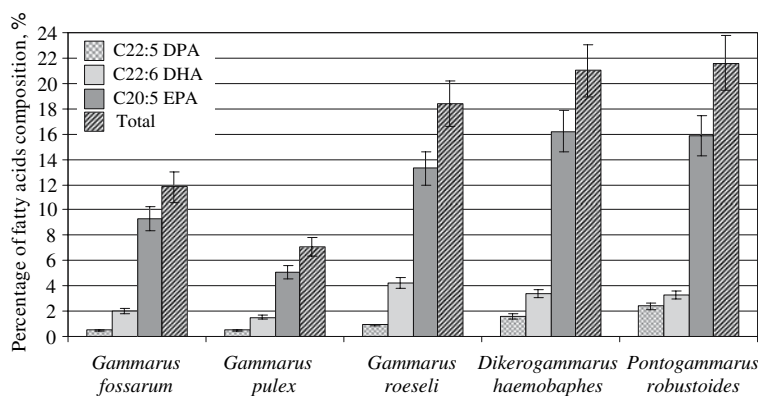
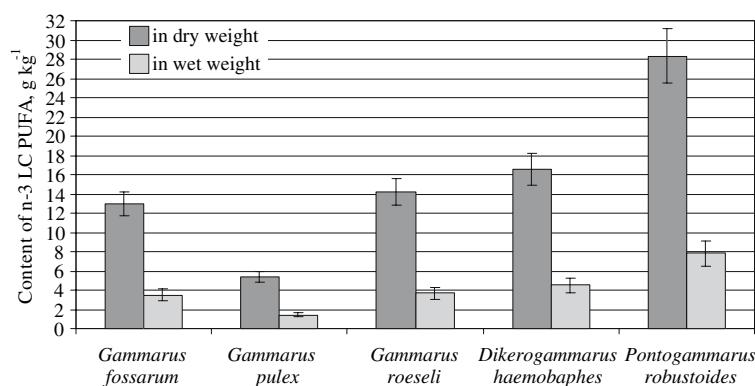
respectively) than in the native ones (0.5% and 1.5–2%, respectively). The levels of DPA and DHA in *G. roeseli* were 0.9 and 4.2%, respectively.

The results obtained showed that the level of n-3 LC PUFA in analyzed gammarids, especially the Ponto-Caspian species, is high and comparable to Antarctic krill and trout and even higher than in some lean fish species (Fig. 5). Comparison of composition of analyzed gammarids to the Antarctic krill (mean of *Euphausia superba* and *Euphausia crystallorophias*) showed much lower (two times) content of the lipid fraction and a similar content of dry weight [15, 27]. Fatty acids composition was comparable in the levels of C16:0 and C22:6 n-3 DHA. The level of C20:5 n-3 EPA was intermediate, i.e., lower in the native gammarid species and higher in the Ponto-Caspian ones than in krill. Total content of saturated and monounsaturated fatty acids was lower, however, polyunsaturated—higher than in krill. Total n-3 LC PUFA content in wet weight was lower in the native gammarids and similar in the Ponto-Caspian species in comparison to krill (Fig. 5).

The results obtained of the gammarids fatty acids examinations were also compared to trout (analyzed in the study) and salmon, tuna and cod (according to the USDA nutrient database data) [28]. Comparison of analyzed gammarids to fatty fish like salmon showed a significantly higher amount of n-3 LC PUFA in salmon due to its very high fat content (200–300 g kg<sup>-1</sup>), whereas fat content of the gammarids was approximately ten times lower. However, the percentage composition of fatty acids showed higher level of n-3 LC PUFA in the Ponto-Caspian gammarid species than in salmon. This was in contrast to lean fish, which contain a higher percentage of n-3 LC PUFA in the fatty acids composition than the analyzed gammarids. These observations are in agreement with others showing that the lower lipid content the higher percentage of n-3 LC PUFA, which are situated in greater extent in membrane phospholipids than in storage lipids [29, 30]. A relatively high percentage of n-3 LC PUFA in the gammarids fatty acid composition suggests that these fatty acids might be situated mainly in tissue cell membranes. Nevertheless, the highest similarities were found

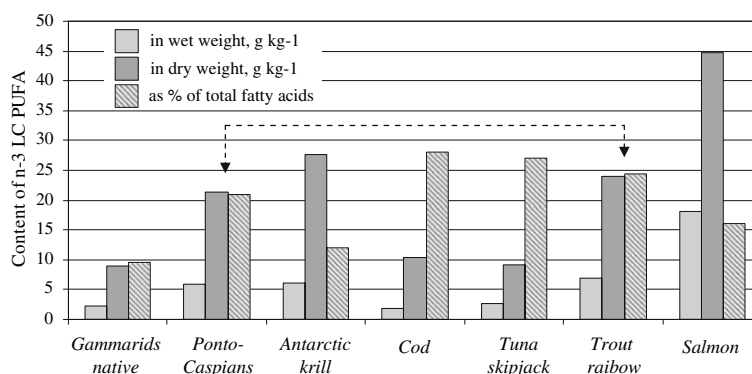
**Table 2** Composition of fatty acids (% of total fatty acids) of analyzed gammarid species compared to wild trout

Fatty acids	<i>G. fossarum</i>	<i>G. pulex</i>	<i>G. roeseli</i>	<i>D. haemobaphes</i>	<i>P. robustoides</i>	Trout wild
C14:0	4.1 ± 0.4	2.2 ± 0.6	1.2 ± 0.3	3.3 ± 1.2	4.2 ± 0.9	3.9 ± 0.4
C15:0	0.5 ± 0.3	0.9 ± 0.1	1.4 ± 0.5	1.2 ± 0.4	1.1 ± 0.3	1.0 ± 0.2
C16:0	17.2 ± 0.4	24.5 ± 1.1	17.3 ± 1.9	19.9 ± 2.0	18.5 ± 2.3	20.4 ± 1.4
C17:0	0.5 ± 0.3	0.7 ± 0.2	1.3 ± 0.4	1.2 ± 0.5	1.4 ± 0.2	0.5 ± 0.2
C18:0	2.9 ± 0.1	5.5 ± 0.2	2.7 ± 0.6	3.5 ± 0.6	3.6 ± 0.7	4.4 ± 0.5
Total SFA	25.2 ± 0.3	33.8 ± 0.4	23.9 ± 0.7	29.1 ± 0.9	28.8 ± 0.8	30.2 ± 0.5
C16:1n-9 + n-7	7.1 ± 1.5	6.7 ± 0.5	5.6 ± 1.3	10.5 ± 2.4	10.1 ± 1.1	7.5 ± 0.8
C18:1n-9	23.2 ± 0.8	28.5 ± 1.1	19.1 ± 1.6	22.1 ± 3.1	20.6 ± 1.0	17.0 ± 0.9
C18:1n-7	3.8 ± 0.5	3.3 ± 0.4	4.1 ± 0.4	4.9 ± 0.6	4.8 ± 0.4	5.8 ± 0.6
C20:1	1.4 ± 0.2	1.0 ± 0.3	1.5 ± 0.5	1.1 ± 0.3	1.4 ± 0.3	2.3 ± 0.5
Total MUFA	35.5 ± 0.6	39.5 ± 0.4	30.3 ± 0.7	38.6 ± 1.2	36.9 ± 0.5	32.6 ± 0.5
C18:2n-6	18.2 ± 0.2	13.0 ± 0.5	15.1 ± 0.6	5.1 ± 0.8	6.2 ± 1.1	8.2 ± 0.9
C18:3n-3	6.4 ± 0.6	4.5 ± 0.2	7.1 ± 0.9	2.2 ± 0.5	2.5 ± 0.3	2.8 ± 0.5
C20:4n-6	2.9 ± 0.2	2.2 ± 0.6	5.2 ± 0.6	3.8 ± 0.4	4.0 ± 0.7	2.3 ± 0.4
C20:5n-3 EPA	9.3 ± 0.8	5.0 ± 1.1	13.3 ± 2.9	16.2 ± 1.3	15.9 ± 1.4	6.9 ± 0.8
C22:5n-3 DPA	0.5 ± 0.3	0.5 ± 0.2	0.9 ± 0.3	1.6 ± 0.2	2.4 ± 0.3	2.2 ± 0.4
C22:6n-3 DHA	2.0 ± 0.2	1.5 ± 0.7	4.2 ± 1.1	3.4 ± 0.5	3.3 ± 0.4	14.8 ± 1.2
Total PUFA	39.3 ± 0.4	26.7 ± 0.6	45.8 ± 1.0	32.3 ± 0.6	34.3 ± 0.7	37.2 ± 0.7
Total n-3 PUFA	18.2 ± 0.4	11.5 ± 0.5	25.5 ± 1.3	23.4 ± 0.6	24.1 ± 0.6	26.7 ± 0.7
Total n-6 PUFA	21.1 ± 0.2	15.2 ± 0.5	20.3 ± 0.6	8.9 ± 0.6	10.2 ± 0.9	10.5 ± 0.6
Ratio n-6:n-3	1:0.86 ± 0.03	1:0.75 ± 0.05	1:1.25 ± 0.1	1:2.62 ± 0.24	1:2.36 ± 0.26	1:2.54 ± 0.25

**Fig. 3** Levels of particular n-3 LC PUFA as percentage of total fatty acids composition of analyzed gammarids**Fig. 4** Content of total n-3 LC PUFA in dry weight and fresh sample of analyzed gammarids



**Fig. 5** Comparison of mean n-3 LC PUFA content in analyzed gammarids, krill and selected fish, similarities between species are marked by arrows



between analyzed Ponto-Caspian gammarid species and trout, especially in the case of total n-3 LC PUFA levels in wet and dry weights, as well as in their percentage of fatty acid composition.

In the study of Makhutova and coworkers [31] it was suggested that gammarids may selectively consume some particles containing EPA from bottom sediments. The selective consumption of n-3 LC PUFA-rich bottom sediment particles by zoobenthos was also reported by Goedkoop and colleagues [32]. Moreover, it was observed that gammarids invading fresh waters of Central and Western Europe, e.g. the Ponto-Caspian species may show predatory behavior, in certain conditions [20]. This can additionally increase the n-3 LC PUFA content in the gammarids' lipid fraction. Graeve and co-workers [15] suggested that Antarctic amphipods can adapt their fatty acids composition to the nutritional condition in both, storage and membrane lipids. Depending on season and food availability they can change their diet. This could be also possible in the case of freshwater amphipods like gammarids and their fatty acid composition may differ depending on season and nutritional conditions. Moreover, Arts and coworkers [16] indicated that the level of EPA and DHA within amphipod tissues could be highest in spring and fall, when food quality (in terms of LC PUFA content) of freshly sedimenting matter is highest because of the inclusion of diatoms, dinoflagellates and cryptomonad

**Table 3** Estimated potential harvest of n-3 LC PUFA, wet and dry gammarids mass in example of *Pontogammarus robustoides* farmed in ponds of varying surface area and 1.5 m depth, assumed mean density ca.  $50 \times 10^3$  animals  $m^{-2}$

Potential harvest	Pond area ( $m^2$ )					
	100	500	1,000	2,500	5,000	10,000
Gammarid wet mass, $t$	0.3	1.5	3.1	7.7	15.5	31
Gammarid dry mass, $t$	0.08	0.4	0.85	2.1	4.2	8.5
Gammarid oil, $t$	0.01	0.05	0.11	0.27	0.55	1.12
n-3 LC PUFA, $t$	0.002	0.01	0.02	0.05	0.12	0.25

algae. A hypothetical model of potential yields of EPA and DHA in prairie ponds of varying surface area was also proposed. These authors supposed that several tons of EPA and DHA could be present in a large pond where amphipods occur at high density. Similar effect might be also expected in the case of gammarids analyzed in this study, especially Ponto-Caspian species. These gammarids are more vital than others occurring in fresh waters of Central and Western Europe and their biomass increases each year [17–19, 21].

It is supposed that the Ponto-Caspian gammarids could be farmed in shallow ponds and might become a innovative and efficient source of n-3 LC PUFA. Depending on pond area and animal density such aquaculture might give several tons of n-3 LC PUFA, especially EPA. As an example *P. robustoides* could be taken. During this study it was shown that this gammarid contains the highest level of n-3 LC PUFA—approximately  $6.9 \text{ g kg}^{-1}$  in the wet weight (i.e.,  $25 \text{ g kg}^{-1}$  in dry weight), among other analyzed ones. It could be estimated that *P. robustoides* farmed in a large pond of 1 ha area and 1.5 m depth, where the mean density is high, assumed as  $50 \times 10^3$  animals  $m^{-2}$ , might give a potential harvest of approximately 1.12 tons of gammarid oil containing high concentration of n-3 LC PUFA, mainly EPA (Table 3). *Pontogammarus robustoides* partial fecundity and number of generation per year are assumed to be 5.1 and 3, respectively, which are the highest among the species analyzed [19]. This suggests that in natural conditions at least three such harvests per year could be obtained.

Various species of gammarids commonly occur in fresh and sea waters worldwide. Due to rapid depletion of sea fish communities [33] farmed gammarids could be regarded as a potentially innovative source of omega-3 LC PUFA rich oil for nutritional, pharmaceutical and animal feeding purposes. Farmed gammarids could be also used for the production of feed for fish aquaculture or for pet food. Under the conditions above one could expect to obtain approximately 31 tons of wet or 8.5 tons of dry gammarid mass for possible use as feed for fish aquaculture.

Moreover, gammarids could be considered as natural and organic feed for farmed trout and other predatory freshwater fish, which in the wild preferably consume these crustaceans.

## References

1. Simopoulos AP (1991) Omega-3 fatty acids in health and disease and in growth and development. *Am J Clin Nutr* 54:438–443
2. Bang HO, Dyerberg J, Nielsen AB (1971) Plasma lipid and lipoprotein pattern in Greenlandic West Coast Eskimos. *Lancet* 7710:1143–1145
3. deDeckere EAM, Korver O, Verschuren PM, Katan MB (1998) Health aspects of fish and n-3 polyunsaturated fatty acids from plant and marine origin. *Eur J Clin Nutr* 52:749–753
4. Banning M (2005) The role of omega-3-fatty acids in the prevention of cardiac events. *Br J Nurs* 25:503–508
5. Ruxton CH, Reed SC, Simpson MJ, Millington KJ (2004) The health benefits of omega-3 polyunsaturated fatty acids: a review of the evidence. *J Hum Nutr Diet* 17:449–459
6. Kris-Etherton PM, Taylor DS, Yu-Poth S, Huth P, Moriarty K, Fishell V, Hargrove RL, Zhao G, Etherton TD (2000) Polyunsaturated fatty acids in the food chain in the United States. *Am J Clin Nutr* 71(S):179–188
7. Sanders TAB (2000) Polyunsaturated fatty acids in the food chain in Europe. *Am J Clin Nutr* 71(S):176–181
8. Simopoulos AP, Leaf A, Salem N (1999) Essentiality of and recommended dietary intakes for omega-6 and omega-3 fatty acids. *Ann Nutr Metab* 43:127–131
9. Kolanowski W, Laufenberg G (2006) Enrichment of food products with polyunsaturated fatty acids by fish oil addition. *Eur Food Res Technol* 222:472–477
10. Trautwein EA (2001) n-3 fatty acids-physiological and technical aspects for their use in food. *Eur J Lipid Sci Technol* 103:45–52
11. Bimbo AP (1998) Guidelines for characterisation of food-grade fish oil. *Inform* 5:180–188
12. Drevon CA (1992) Marine oils and their effects. *Nutr Rev* 4:38–45
13. Jezierska B, Hazel JR, Gerking SD (1982) Lipid mobilization during starvation in the rainbow trout, *Salmo gairdneri* Richardson, with attention to fatty acid. *J Fish Biol* 21:681–692
14. MacNeil C, Elwood RW, Dick JTA (2000) Factors influencing the importance of Gammarus spp. (Crustacea: Amphipoda) in riverine salmonid diets. *Arch Hydrobiol* 149:87–107
15. Graeve M, Dauby P, Scailteur Y (2001) Combined lipid, fatty acid and digestive tract content analysis: a penetrating approach to estimate feeding models of Antarctic amphipods. *Polar Biol* 24:853–862
16. Arts MT, Ackman RG, Holub BJ (2001) “Essential fatty acids” in aquatic ecosystems: a crucial link between diet and human health and evolution. *Can J Fish Aquat Sci* 58:122–137
17. Bij de Vaate A, Jazdzewski K, Ketelaars H, Gollasch S, Van der Velde G (2002) Geographical patterns in range extension of macroinvertebrate Ponto-Caspian species in Europe. *Can J Fish Aquat Sci* 59:1159–1174
18. Bacela K, Konopacka A (2004) The life history of *Pontogammarus robustoides*, an alien amphipod species in Polish waters. *J Crustacean Biol* 25:190–195
19. Jazdzewski K, Konopacka A, Grabowski M (2002) Four Ponto-Caspian and one American gammarid species (Crustacea, Amphipoda) recently invading Polish waters. *Contrib Zool* 71:115–122
20. Grabowski M, Bacela K, Konopacka A (in press) How to be an invasive gammarid; comparison of life history traits. *Hydrobiologia*
21. Grabowski M (2006) Rapid colonization of the Polish Baltic coast by an Atlantic palaemonid shrimp *Palaemon elegans* Rathke, 1837. *Aquat Invasions* 3:116–123
22. Schöll F (2003) Makrozoobentos Odry 1998–2001. Miedzynarodowa Komisja Ochrony Odry przed Zanieczyszczeniem, Wrocław
23. Schmidt U (1999) Das Makrozoobenthos des Unteren Odertals–Faunenzusammensetzung und siedlungsdynamik in einer Flußbaue. *Limnologie aktuell* 9:317–336
24. Nicol S, Foster J (2003) Recent trends in the fishery for Antarctic krill. *Aquat Living Resour* 16:42–45
25. Folch J, Lees M, Sloane-Stanley GH (1957) A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem* 226:497–509
26. American Oil Chemists’ Society (1997) Official method Ce 1b-89 fatty acid composition by GLC-marine oils (modified), official methods and recommended practices of the AOCS, 5th edn. AOCS, Champaign
27. Ju SJ, Harvey HR (2004) Lipids as markers of nutritional condition and diet in the Antarctic krill *Euphausia superba* and *Euphausia crystallorophias*. *Deep-Sea Res II* 51:2199–2214
28. United States Department of Agriculture (accessed May 2006) USDA national nutrient database for standard release. <http://www.nal.usda.gov>
29. Sushchik NN, Gladyshev MI, Moskvichova AV, Makhutova ON, Kalachova GS (2003) Comparison of fatty acid composition in major lipid classes of the dominant benthic invertebrates of the Yenisei river. *Compar Biochem Physiol Part B* 134:111–122
30. Williams EE, Anderson MJ, Miller TJ, Smith SD (2004) The lipid composition of hypodermal membranes from the blue crab (*Callinectes sapidus*) changes during the molt cycle and alters hypodermal calcium permeability. *Compar Biochem Physiol Part B* 137:235–245
31. Makhutova ON, Kalachova GS, Gladyshev MI (2003) A comparison of the fatty acid composition of *Gammarus lacustris* and its food sources from a freshwater reservoir, Bugach, and the saline Lake Shira in Siberia, Russia. *Aquat Ecol* 37:159–167
32. Goedkoop W, Sonesten L, Ahlgren G, Boberg M (2000) Fatty acids in profundal benthic invertebrates and their major food resources in Lake Erken, Sweden: seasonal variation and trophic indications. *Can J Fish Aquat Sci* 57:2267–2279
33. Myers RA, Worm B (2003) Rapid worldwide depletion of predatory fish communities. *Nature* 423:280–283