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Loss of Nutritionally Essential n-3 PUFA in Riverine Benthic Macroinvertebrates Following an Extreme Rainfall Event

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Abstract

The Anastasia River (southern Sakhalin Island) is a key salmon spawning ground, where summer storm floods can drastically alter benthic communities that form the diet of fish. This study assessed the impact of heavy rainfall on the benthic macroinvertebrates in the lower reaches of the river by analyzing taxonomic composition, biomass, and fatty acid (FA) profiles of dominant taxa before and after a flood event. A catastrophic decline in biomass was observed (from 35.7 ± 4.4 g m^{-2} to 1.74 ± 0.68 g m^{-2}), alongside a significant shift in community structure. Crustaceans (dominated by *Eogammarus kygi*), with a unique FA profile rich in long-chain n-3 and n-6 polyunsaturated fatty acids (PUFAs), were the primary bearers of high nutritional value. All crustaceans exhibited omnivorous diets, with river crabs (*Eriocheir japonica*) having a broader spectrum than conspecifics inhabiting the marine littoral zone. Amphipods were key processors of allochthonous matter. The flood caused not only a quantitative but also a severe qualitative reduction in community nutritional value, with the content of physiologically crucial n-3 and n-6 PUFAs dropping by a factor of 25 and 15 on average, respectively. The disproportionately high loss of n-3 PUFAs indicates that the qualitative degradation of food resources by extreme floods may be an underestimated factor limiting the post-flood recovery of fish populations.

Keywords: salmonid river; flood; benthic food web; diet; fatty acid trophic markers; allochthonous organic matter; food quality; EPA; DHA

1. Introduction

In small river ecosystems, the trophic web is based on periphyton, which is supplemented to varying degrees by leaf litter [1–3]. This autochthonous and allochthonous organic matter (OM) is subsequently consumed by benthic invertebrates [4–6], which form the food base for ichthyofauna. The survival and growth of fish directly depend on the abundance, composition, and nutritional value of food resources. Terrestrial invertebrates can make a substantial contribution, in addition to benthic invertebrates, to fish diets, especially in small shaded rivers under conditions of limited primary food resources [7,8]. However, terrestrial invertebrates contain considerably lower levels of polyunsaturated fatty acids (PUFAs) in general and eicosapentaenoic acid (EPA, 20:5n-3) in particular, while having higher levels of linoleic acid (LIN, 18:2n-6) compared to aquatic ones [9,10]. Moreover, docosahexaenoic acid (DHA, 22:6n-3) is completely absent in terrestrial species [10,11].

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Thus, periphyton and benthic invertebrates remain the key source of physiologically valuable long-chain PUFAs (LC-PUFAs) of the n-3 family, namely EPA and DHA, and other high-value compounds [6,12].

Aquatic ecosystems play a unique role in the biosphere as the primary source of EPA and DHA, which are of high physiological value for most animals [13–16]. EPA primarily serves as a substrate for the synthesis of eicosanoids (hormone-like substances), specifically, prostaglandin-3 (PGI_3, PGE_3, and PGH_3), thromboxane-3 (TXA_3 and TXB_3), leukotriene-5 (LTB_5), and resolvins (PE_1 and PE_2) [17–19]. Eicosanoids serve to regulate cardiovascular function, immune response, blood clotting and inflammatory processes. DHA plays a structural role in membranes, especially in neural tissue cells [20,21], and serves as a substrate for the synthesis of various resolvins, protectins, and maresins [22,23]. The prolonged absence of EPA and DHA, and additionally arachidonic acid of n-6 family (ARA, 20:4n-6) in diets leads to various pathologies: myocarditis, hepatic and intestinal steatosis, gill hemorrhage, fin erosion, spinal curvature, impaired immune response, etc. [24–26]. At the population level, this results in disrupted feeding behavior (increased risk of predation), decreased growth rate, and increased fish mortality [25,27]. Alpha-linolenic acid (ALA, 18:3n-3) and LIN are also physiologically important, as they serve as essential substrates for the synthesis of n-3 and n-6 LC-PUFAs in consumers [28,29].

The small rivers of southern and southeastern Sakhalin, with predominantly mountain-foothill type of channels, are key spawning areas for Pacific salmons, primarily pink (*Oncorhynchus gorbuscha*) and chum (*Oncorhynchus keta*) salmon [30]. The estuaries and lower reaches of these small rivers play a critical role in the adaptation of juveniles during their transition to the marine stage of life, as the success of smoltification depends on their hydrological regime and the quantity and biochemical quality of food resources, e.g., Refs. [31,32]. In addition to anadromous species, South Sakhalin rivers are home to a wide range of typically freshwater species that also depend on benthic fauna [33,34].

Under climate change, extreme weather events such as intense rainstorms are becoming more frequent, with some regions experiencing a pronounced increase in the intensity of short-term extreme precipitation [35]. Such regions include southern Sakhalin and the Russian Far East in general, where extreme weather events occur at higher rates [36,37]. These events lead to a rapid rise in water levels, increased current velocity and turbidity, and the restructuring of bottom substrates and benthic macroinvertebrate communities [38].

The impact of floods on the structure of benthic macroinvertebrate communities is being actively studied. In most rivers, intense rainstorms lead to a decline in species richness, density, and biomass of benthic invertebrates due to high flow velocity, substrate mobility and erosion, coupled with a drop in dissolved oxygen and changes in nutrient concentrations [39–41]. In small mountain and foothill rivers, similar to many in southern Sakhalin, extreme weather events are particularly hazardous, as they can cause abrupt changes in benthic communities, and recovery occurs more slowly than in low-gradient rivers [40,42]. Several studies describe the structural and functional reorganization of benthic macroinvertebrate communities after floods, e.g., Refs. [42,43], but there is a lack of research linking these changes to the nutritional value of benthic macroinvertebrates for fish. There are no data on changes in the biochemical quality of the fish food base, for example, on changes in the content of n-3 PUFAs, which are key nutrients for fish growth and development. Nevertheless, it is well known that the nutritional value of benthic invertebrates mainly depends on phylogenetic and trophic factors [44–46].

In this study, we assessed changes in the taxonomic composition and biomass of benthic macroinvertebrates before and after a rain-induced flood in a typical river in southern Sakhalin, the Anastasia River. The primary cause of these changes during the first days after the flood was presumably mechanical washout (i.e., the physical removal of organisms by increased water flow and bedload movement), rather than changes in water chemistry, as supported by the findings of other studies, e.g., Refs. [47,48]. We analyzed the qualitative and quantitative composition of the fatty acid profile in dominant taxa, characterized the trophic structure of the benthic macroinvertebrate community based on FA profiles, and quantitatively estimated the loss of nutritional value (primarily physiologically valuable PUFAs) in benthic macroinvertebrates for fish following an extreme hydrological event. This study provides the first assessment (from taxonomy to biochemistry) of the consequences of rainstorm floods for the food base of Sakhalin's salmon rivers in the context of climate change.

2. Materials and Methods

2.1. Study Area, Hydrological Event, and Environmental Parameters

The study was conducted on the Anastasia River, located in Aniva Bay on Sakhalin Island and flowing into the Sea of Okhotsk. The river is 16 km long, with a catchment area of 24.1 km^2 and an average water discharge of 0.43 m^3 s^{-1} [49]. The river is a spawning ground for salmonids. Samples for studying the taxonomic composition, density, biomass, and fatty acid composition and content of benthic macroinvertebrates were collected along a 2.5 km river section. Sampling sites (Figure 1) were located at 500 m and 1.5–3 km upstream of the mouth. Sampling at the river mouth area was carried out before the rainstorm event, and in the lower reaches of the river, several days before the rainstorm and after it ended.

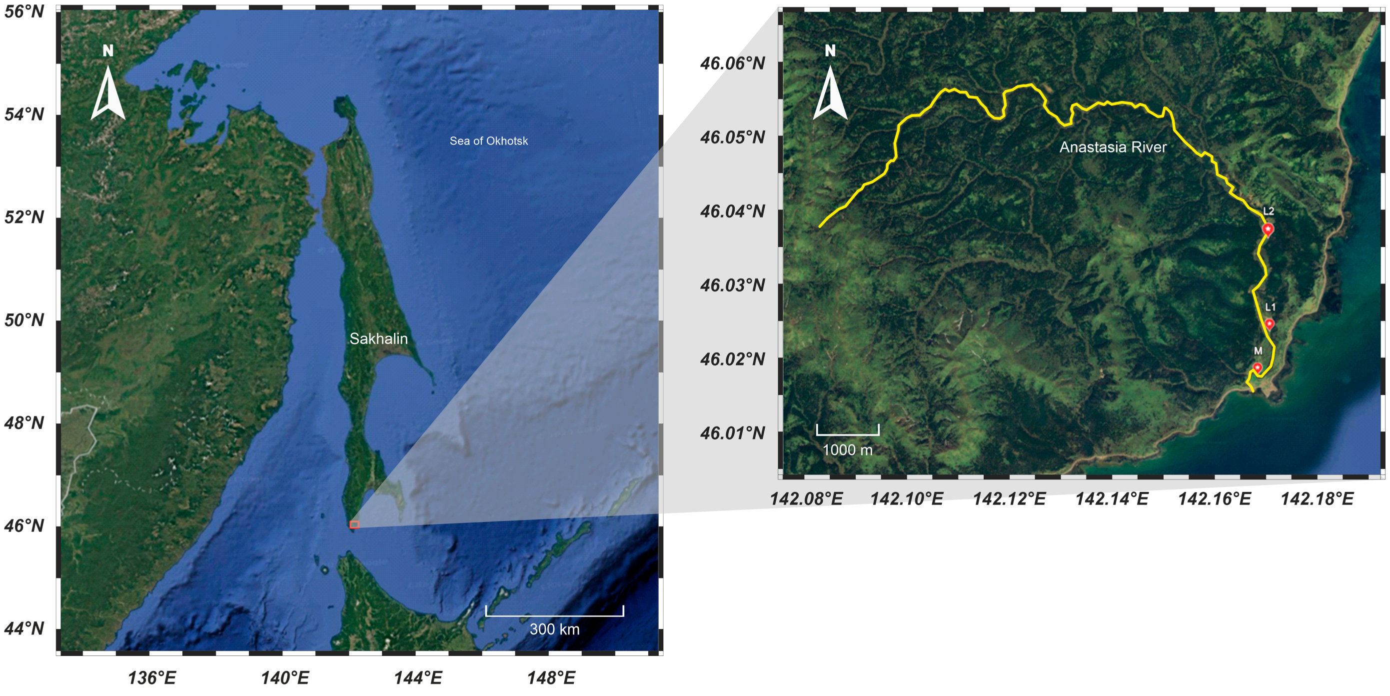

Figure 1. Map of sampling sites in the Anastasia River, Sakhalin Island, Russia. Sampling points: M—the river mouth (46.01548° N, 142.16983° E), sampled before the rainstorm event (4–6 August); L1–L2—the lower reaches of the river (the area from 46.02405° N, 142.17975° E to 46.03754° N, 142.16996° E), sampled before the rainstorm (4–6 August) and after it ended (11–12 August).

According to observations from the "Cape Crillon" meteorological station (Hydrometeorological report No 7-3-41, Sakhalin Administration for Hydrometeorology and Environmental Monitoring, available at https://doi.org/10.5281/zenodo.19060043 accessed on 17 March 2026), due to the intensification of frontal boundaries, 100.8 mm of precipitation fell from 8 p.m. on 7 August to 8 a.m. on 8 August. This constituted 99% of the monthly precipitation norm. The very heavy rain was accompanied by very strong winds. Using the limiting intensity method, we estimated that the river's discharge could have increased by more than two orders of magnitude (excluding losses due to absorption, evaporation, and retention). The river mouth before the flood and the day after is shown in Figure S1.

The substrate along the study section consisted of large pebbles with boulders, very slightly silted, with weak or absent biofilms on the stones. The water temperature during the study period varied between 14 and 22 °C. Measurements of pH (6.5 ± 0.17) and total dissolved solids (TDS: 57.3 ± 5.55 mg L^{-1}) were conducted using an HI 98129 Combo (Hanna Instruments, Woonsocket, RI, USA) meter ten days after the end of the rainstorm to provide a general characterization of the river conditions.

2.2. Benthic Macroinvertebrate Sampling and Processing

Samples for the analysis of taxonomic composition, density, and biomass (wet weight) of benthic macroinvertebrates were collected using a Surber sampler (213 µm mesh size) in 3 to 6 replicates per site. Each replicate covered an area of 0.16 m^2. Organisms were extracted from the samples and preserved in 96% ethanol. Identification, counting, and weighing were conducted using binocular microscopes (Olympus CX 41, Olympus Corporation, Tokyo, Japan; MBS-10, Lytkarino Optical Glass Factory, Lytkarino, Russia) and torsion balances (WT-500, Techniprot, Pruszków, Poland), respectively.

Additional benthic macroinvertebrate samples were collected for biochemical analysis. For this purpose, dominant (by biomass) groups were selected from the samples, sorted by large taxa, weighed, and placed in a chloroform-methanol mixture (2:1 by volume) for further fatty acid analysis. The number of individuals per sample ranged from 5 to 50 for most taxa, with the exception of Japanese mitten crabs (*Eriocheir japonica*), which comprised 2–3 individuals per sample due to their larger size. The samples were stored at −20 °C until subsequent biochemical processing. For fatty acid analysis, Japanese mitten crabs were collected from both the marine littoral zone and the river. This species is catadromous, migrating between freshwater and marine environments during its life cycle [50]. Specimens from the marine littoral zone were therefore sampled to assess the dietary plasticity of the species across these different habitats, providing a comparative basis for understanding potential shifts in trophic strategies.

2.3. Fatty Acid Analysis

Fatty acid analysis included the homogenization and lipid extraction, preparation of fatty acid methyl esters (FAMEs), and mass spectrometry analysis. The procedures followed Christie and Han [51]. Briefly, samples were homogenized mechanically in a porcelain mortar with the addition of glass beads. Lipids were extracted with a mixture of chloroform and methanol (2:1 by volume), distilled water was added, and the water–methanol phase was removed. The resulting extracts were dried by passing through a layer of anhydrous Na_2SO_4. Chloroform was evaporated using a rotary vacuum evaporator equipped with a water bath and cryostat (IKA RV8, IKA HB10, IKA RC2 control, IKA, Staufen, Germany) at 35 °C. FAMEs were obtained by acid-catalyzed transmethylation using a solid-state thermostat (Dry Block Heater 1, IKA, Staufen, Germany). A double volume of distilled water and 200 µL of hexane were added to the mixture, shaken vigorously for 2–3 min. After that, the FAMEs were purified to remove impurities using thin-layer chromatography and were eluted from the silica gel using chloroform.

Quantitative analysis of FAMEs was performed using a gas chromatograph with a flame ionization detector (Shimadzu GC 2010 Plus, Shimadzu, Kyoto, Japan). A 30-m capillary column, Supelcowax 10, with an internal diameter of 0.25 mm was used for the quantitative analysis. For precise identification of FAMEs, a gas chromatograph coupled with a mass spectrometric detector (Shimadzu GCMS-QP2010 Ultra, Shimadzu, Kyoto, Japan) was additionally used. Fatty acid peaks were identified by comparing the obtained mass spectra with those available in the Agilent databases (Wiley, NIST) and by comparing retention times with those of commercial standards (Supelco 37 Component FAME Mix: Sigma-Aldrich Co., St. Louis, MO, USA). The relative content of FAs was determined as the ratio of the peak area of a specific FA to the sum of the areas of all FA peaks. The absolute content of fatty acids was calculated based on the peak area, which corresponded to a known quantity of the internal standard (a solution of 19:0 methyl ester in chloroform, 0.5 mg mL^{-1}; Nu-Check-Prep, Inc., Elysian, MN, USA) added to each sample prior to the biochemical procedures.

2.4. Assessment of the Diet of Benthic Macroinvertebrates

The diets of benthic macroinvertebrates were assessed using the analysis of FA-markers. This biochemical method has undergone extensive validation and is widely applied in trophic ecology [52,53]. Bacterial and plant taxa, including microalgae, have a specific set of enzymes and are capable of synthesizing specific fatty acids. When consumed, these FAs are incorporated into consumer tissues with minimal modification, thus carrying information about their dietary origin and serving as reliable biomarkers. A key advantage of this method is its ability to evaluate the assimilated portion of the diet, including visually unidentifiable components [53].

2.5. Quantitative Assessment of PUFA Content and Nutritional Value of the Benthic Community

To determine the nutritional value of benthic macroinvertebrates in the river mouth and lower reaches of the river and to assess the impact of rainfall flood on changes in the nutritional value of the community, the content of all essential and physiologically valuable n-3 PUFAs and n-6 PUFAs was calculated. The concentration (mg g^{-1} wet weight) of FAs was assessed using an added internal standard. Data on the biomass of each macroinvertebrate taxa and the concentration of FAs in benthic macroinvertebrate taxa collected at each station were used. This approach made it possible to calculate the total pool of essential PUFAs available in the benthic communities per unit area before and after the flood event.

2.6. Statistical Analysis

The normality of the data was checked using the Shapiro–Wilk test. Because of unequal variances (Bartlett's test, $p < 0.05$), we used Welch's ANOVA to test for differences in contents of 18:2n-6, 20:4n-6, 18:3n-3, 20:5n-3, 22:6n-3, and n-3, and n-6 PUFAs, (mg m^{-2}) before rainfall flood at the river mouth area, before the flood in the lower reaches, and after the flood in the lower reaches, followed by the Games–Howell post hoc test for pairwise comparisons. All *p*-values were corrected for multiple testing using the Games–Howell procedure.

To visualize the separation among taxonomic groups and sampling sites and to identify the contribution of individual fatty acids, we performed a principal component analysis (PCA). Crabs were excluded from this analysis due to their ability to move freely over large distances within the river channel.

Hierarchical cluster analysis for FA profiles of benthic macroinvertebrates was performed using Euclidean distances with Ward's clustering method. To reduce multicollinearity, FA variables were screened prior to analysis: pairwise Pearson correlations were calculated, and in cases where the correlation coefficient exceeded 0.75, one variable from each correlated pair was excluded. Of the 39 initial FAs, 12 were removed, and the remaining 27 were used in the final analysis.

For pairwise comparisons of FAs in crabs from the river and the marine littoral zone, an independent two-tailed Student's *t*-test was used when the data were normally distributed and had homogeneous variances. The Mann–Whitney *U* test was applied when the data exhibited non-normality and heterogeneous variances.

Data processing and visualization were performed using RStudio (version 4.5.1) and Microsoft Excel. Figures were edited in CorelDRAW (version 24.3).

3. Results

3.1. Taxonomic Composition and Community Structure

A total of 39 taxa of benthic macroinvertebrates were identified in the Anastasia River, including 14 species and higher taxa of Diptera, 8 species of Ephemeroptera, 7 species of Trichoptera, 3 species each of Crustacea and Oligochaeta, 2 species of Plecoptera, and 1 species each of Megaloptera and Coleoptera (Table S1).

The communities were dominated by crustaceans (Table 1). Since crabs (*E. japonica*) are large and mobile, different sampling gear would be required for an accurate assessment of their density and biomass. Nevertheless, even the obtained results indicate their high density and biomass in the river (Table 1). Therefore, further community analysis was conducted excluding crabs.

Table 1. Average (M ± SE) density (ind. m^{-2}) and biomass (mg m^{-2}) of the main taxonomic groups of benthic macroinvertebrates collected in the Anastasia River, Sakhalin Island, August 2025.

Taxa	**River Mouth**	**Lower Reaches**	
		Before Flood	**After Flood**
Density, ind. m^{-2}			
Amphipoda	1771 ± 536	6343 ± 938	140 ± 32
Ephemeroptera	66 ± 37	0	42 ± 13
Trichoptera	48 ± 17	88 ± 38	49 ± 34
Diptera	66 ± 29	73 ± 39	48 ± 9
Plecoptera	51 ± 35	7 ± 7	7 ± 5
Megaloptera	4 ± 4	0	0
Coleoptera	0	15 ± 7	3 ± 2
Oligochaeta	44 ± 15	0	1 ± 1
Total *	2050 ± 538	6527 ± 882	290 ± 70
Decapoda, *Eriocheir japonica*	15 ± 7	15 ± 15	1 ± 1
Biomass, mg m^{-2}			
Amphipoda	14,245 ± 3778	34,492 ± 4808	1142 ± 306
Ephemeroptera	87 ± 56	0	60 ± 25
Trichoptera	230 ± 117	414 ± 178	496 ± 240
Diptera	345 ± 203	804 ± 769	76 ± 24
Plecoptera	48 ± 34	8.8 ± 8.8	7.3 ± 4.8
Megaloptera	1.5 ± 1.5	0	0
Coleoptera	0	11.7 ± 7.8	1.4 ± 1.3
Oligochaeta	5.5 ± 3.8	0	0
Total *	14,961 ± 3746	35,730 ± 4419	1783 ± 407
Decapoda, *Eriocheir japonica*	11,627 ± 5686	61,173 ± 61,173	1210 ± 1210

* The density and biomass of *Eriocheir japonica* are excluded from the total invertebrate count.

Amphipoda were represented by two species, with the larger one, *E. kygi*, dominating. At the river mouth area and in the lower reaches, before the flood, amphipods constituted an average of 86–97% of total abundance and 95–97% of total biomass. In the lower reaches, after the rainfall, their abundance and biomass decreased to 48% and 64%, respectively, while the proportion of caddisflies, mayflies, and dipterans in the community increased

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(Figure 2). On average, after the flood, the density and biomass of benthic macroinvertebrates in the lower reaches of the river were 23-fold and 20-fold lower, respectively, than before the rainfall. These changes were primarily driven by a sharp decline in Amphipoda, whose density and biomass decreased by a factor of 45 and 30, respectively. Substantial changes were also observed for Coleoptera and Diptera. Coleoptera density decreased 5-fold, while its biomass declined 10-fold. For Diptera, the change in density was negligible, but biomass decreased by a factor of 10. Crab density and biomass declined 15-fold and 50-fold, respectively (Table 1).

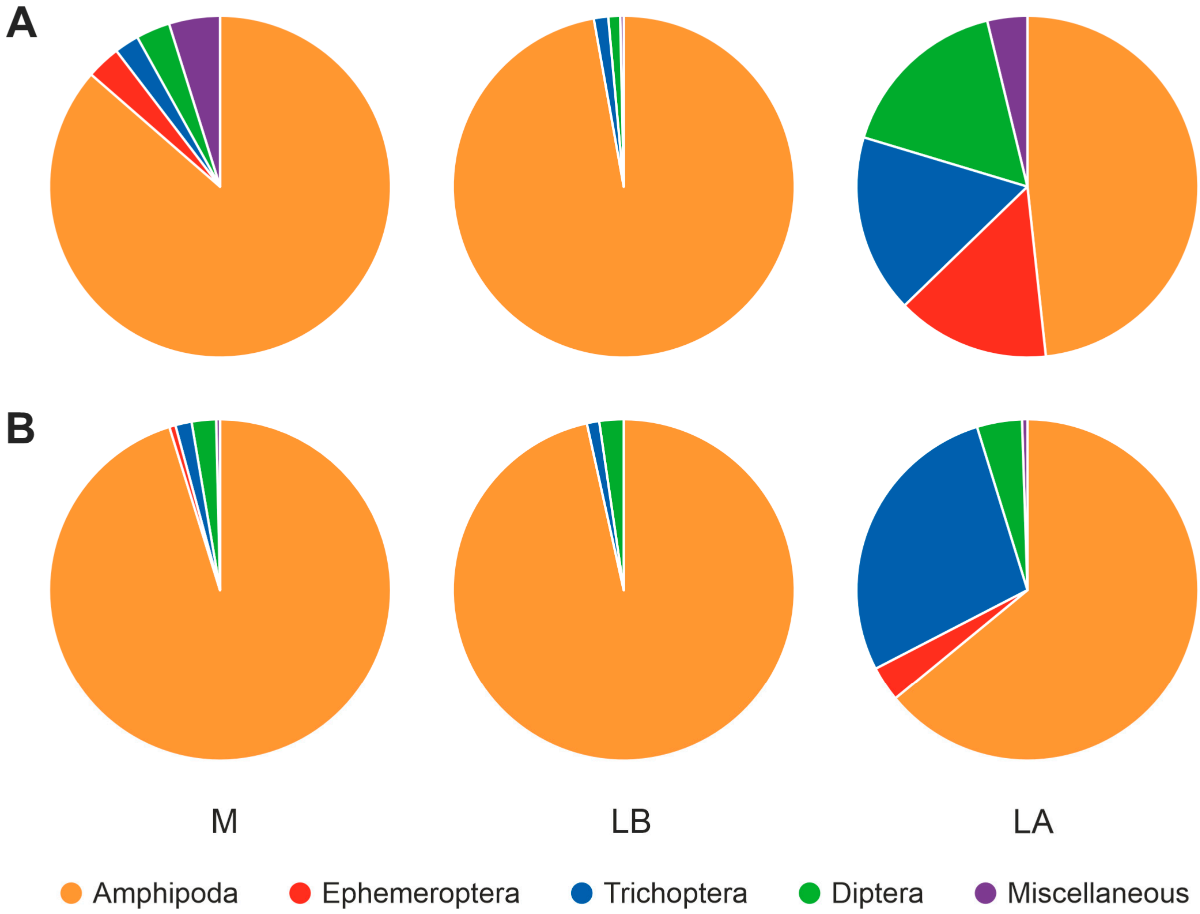

Figure 2. Proportional abundance (**A**) and biomass (**B**) (% of total) of taxonomic groups of benthic macroinvertebrates, excluding *Eriocheir japonica*, before the flood at the river mouth area (M), before the flood in the lower reaches (LB), and after the flood in the lower reaches (LA) of the Anastasia River, Sakhalin Island, August 2025.

Before the flood in the lower reaches and the river mouth, juvenile amphipods (<7 mm) dominated, comprising 60% and 80% of the total biomass and abundance, respectively. After the flood, the proportion of juvenile amphipods decreased to 15% of biomass and 58% of abundance. The proportion of large individuals ($\geq$7) doubled after the flood event.

3.2. Fatty Acid Profiles and Nutritional Value of Dominant Taxa

Over 70 FAs were detected in Amphipoda, Decapoda, Trichoptera, Ephemeroptera, Diptera, and Plecoptera. PUFAs (34.0–43.4%) dominated in all taxa, except in one stonefly. The contents of saturated fatty acids (SFAs; 27.3–34.4%) and monounsaturated fatty acids (MUFAs; 28.8–38.3%) were similar and slightly lower than that of PUFAs (Table S2).

Fatty acid profiles exhibited taxon-specific characteristics. Crustaceans were characterized by high percentages of all C20 MUFAs (20:1n-11, 20:1n-9, and 20:1n-7), PUFAs of the n-6 family (LIN, 20:2n-6, ARA, and 22:5n-6), and LC-PUFAs of the n-3 family (22:5n-3 and DHA). FA profiles of amphipods and crabs were similar, although crabs showed greater variability. In crabs, for instance, levels of LIN and ALA varied within 4.2–23.7% and 2.4–9.2%, respectively, versus 5.7–12.4% and 5.0–7.2% in amphipods.

In all insect larvae, C22 PUFAs, including physiologically valuable DHA, were either absent or present only in trace amounts. Non-case-building caddisflies (*Glossosoma* sp., *H. orientalis*, *S. marmorata*) were characterized by high relative percentages of short-chain MUFAs and PUFAs (C12 and C14 MUFAs, C12 and C14 PUFAs), 16:4n-3 (a marker for green microalgae), and ALA (a marker for green microalgae, cyanobacteria, and aquatic macrophytes). Caddisflies of the genus *Goera* sp. (case-building species) had the highest content of 14:0, 16:0, 16:1n-7, C16 PUFA (markers of green and diatom algae), 18:3n-6 (marker of some species of cyanobacteria), and 18:4n-3 (a marker of cryptophytes). Mayflies were characterized by high levels of C16 MUFA (16:1n-9, 16:1n-7, 16:1n-5), 18:1n-7, ALA, and 18:4n-3. Dipterans exhibited high variability in FA composition, with LIN levels ranging from 5.2% to 26.9%. This FA was the highest in dipterans. *Leuctra* sp. and *Sweltsa* sp. (P1) contained the highest levels of 13:0, i15:0, i17:0, ai17:0 (bacterial markers) and 18:1n-9, whereas P2 (species undetermined) contained the highest level of ALA (Table S2).

The total FA content ranged from 13.1 mg g^{-1} of wet weight (WW) in crabs to 29.0 mg g^{-1} WW in mayflies (Table S2). The concentration of LIN ranged from 0.5 mg g^{-1} WW in caddisflies to 1.9 mg g^{-1} WW in dipterans. The concentration of ALA ranged from 0.8 mg g^{-1} WW in crustaceans to 3.5 mg g^{-1} WW in mayflies. The concentration of ARA ranged from 0.1 mg g^{-1} WW in caddisflies to 0.6 mg g^{-1} WW in amphipods. The concentration of EPA ranged from 1.5 mg g^{-1} WW in caddisflies to 2.6 mg g^{-1} WW in mayflies. The maximum concentration of DHA was found in amphipods and amounted to 0.5 mg g^{-1} WW. In all insect taxa, DHA was present only in trace amounts. The n-3/n-6 ratio was highest in caddisflies and mayflies and lowest in dipterans, but was above 1 in all taxa.

3.3. Multivariate Analyses of Fatty Acid Composition

The benthic macroinvertebrates were visualized in the two-dimensional space defined by the first two principal components based on their fatty acid composition (Figure 3). Factor 1, explaining 31.1% of the total variance, represented a gradient from fatty acids with negative scores (ALA, i13:0, 12:0, C12 and C14 MUFAs, and C12 and C14 PUFAs) to those with positive scores (22:5n-3, 20:1n-7, DHA, 22:5n-6, 20:1n-11, 20:1n-9, 15:0, and ARA). Factor 2, explaining 17.1% of the total variance, represented a gradient from fatty acids with negative scores (22:0, 20:0, and 17:0) to those with positive scores (16:3n-3, 16:0, 16:2n-6, 18:3n-6, and 16:1n-7) (Figure 3).

Amphipods were spatially separated from non-case-building caddisflies along Factor 1, while case-building caddisflies (*Goera* sp.) and mayflies were separated from non-case-building caddisflies along Factor 2 (Figure 3). The most homogeneous FA profiles were observed in amphipods, which formed the densest cluster, while caddisflies exhibited the most heterogeneous FA profiles. No association was found between the FA composition of the taxa and the distance of the collection sites from the river mouth.

Based on fatty acid composition, amphipods from the river mouth area were separated from those collected further upstream. Amphipods collected at the maximum distance from the mouth showed a 2-fold higher level of LIN and a 1.5-fold lower level of EPA and DHA compared to those from the river mouth area. Caddisflies, excluding *Goera* sp., and dipterans tended to form distinct clusters, while mayflies and stoneflies were highly variable and grouped into different clusters (Figure 4). *Goera* sp. formed a distinct cluster with two samples of mayflies. Three caddisfly samples formed a separate subcluster and a tight grouping in the PCA (Figures 3 and 4). Their FA profiles were characterized by high levels of 18:0, 20:0, and 22:0.

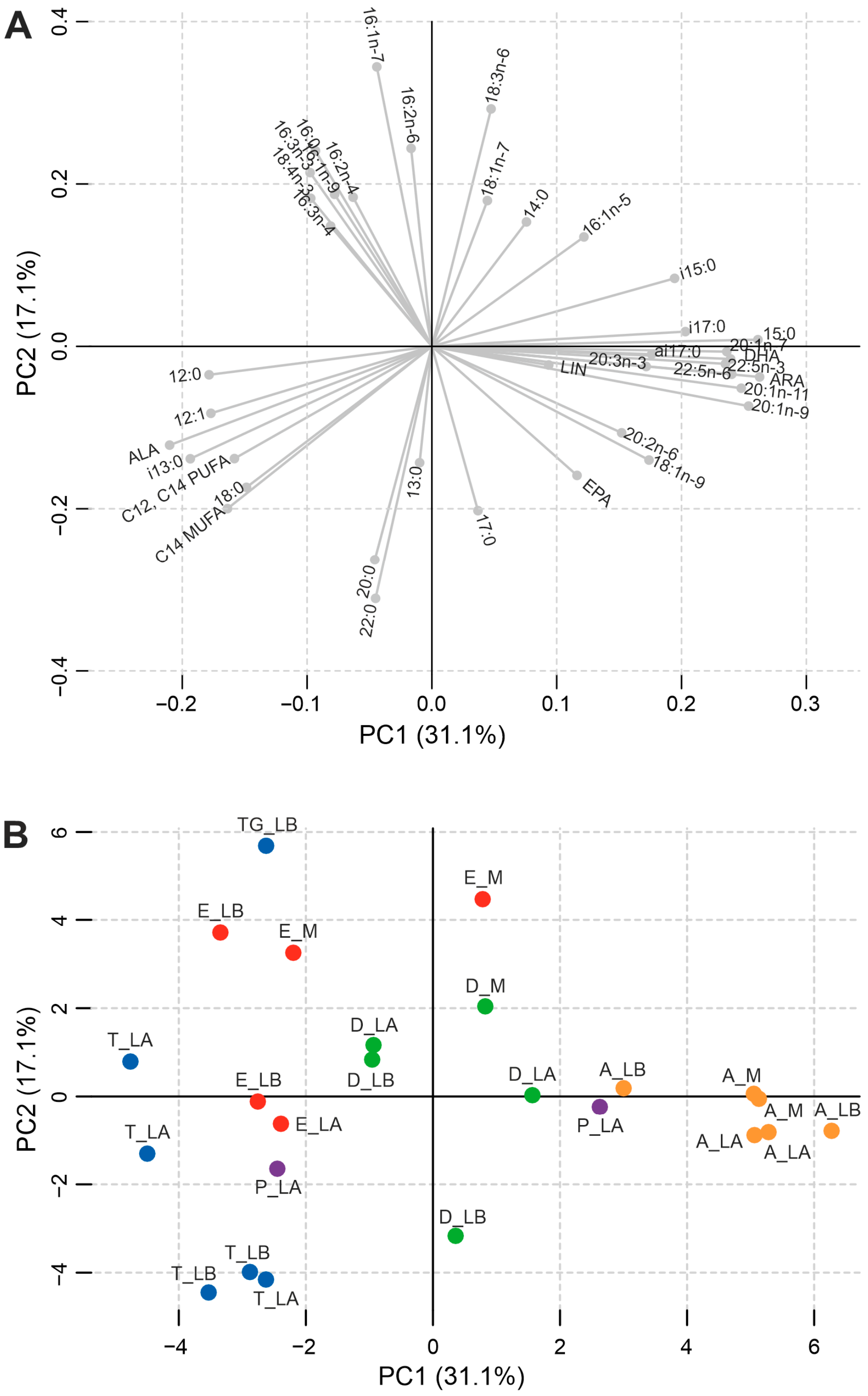

Figure 3. Principal component analysis (PCA) biplot visualizing the relative percentages of fatty acids (**A**) in benthic macroinvertebrates (**B**) from the Anastasia River, Sakhalin Island, August 2025. PC1 and PC2 explain 31.1% and 17.1% of the total variance, respectively. Symbols: A (orange)—amphipods, P (purple)—stoneflies, E (red)—mayflies, T (blue)—non-case-building caddisflies, TG (blue)—*Goera* sp. caddisflies, D (green)—dipterans. Sampling area: M—the river mouth; LB—the lower reaches before the flood; LA—the lower reaches after the flood. Fatty acid abbreviations: linoleic acid (LIN, 18:2n-6), arachidonic acid (ARA, 20:4n-6), eicosapentaenoic acid (EPA, 20:5n-3), and docosahexaenoic acid (DHA, 22:6n-3).

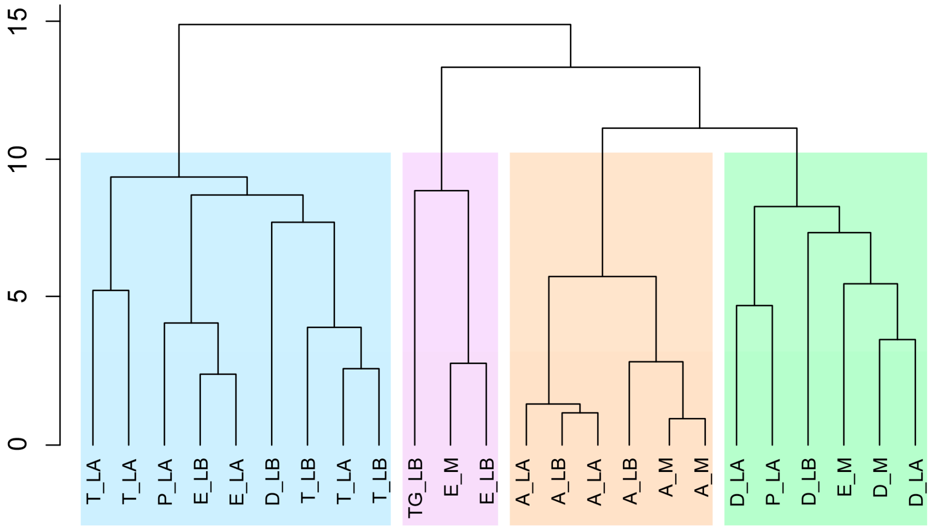

Figure 4. Dendrogram of cluster analysis of the relative percentage of fatty acids in benthic macroinvertebrates of the Anastasia River, Sakhalin Island, August 2025. Symbols: A—amphipods, P—stoneflies, E—mayflies, T—non-case-building caddisflies, TG—*Goera* sp. caddisflies, D—dipterans. Sampling area: M—the river mouth; LB—the lower reaches before the flood; LA—the lower reaches after the flood. Shaded areas highlight the main clusters.

3.4. Impact of the Flood on the Nutritional Value of the Benthic Community

The content of LIN, ARA, ALA, EPA, and DHA, as well as the sums of n-3 and n-6 PUFAs, were quantified for the benthic macroinvertebrate community (mg m^{-2}) at each river section (Figure 5). The intense and prolonged rainfall significantly reduced the PUFA content in benthic macroinvertebrate communities. The lowest nutritional value of the community for all measured FAs was recorded after the flood. The differences in the lower reaches of the river before and after the rain event for individual PUFAs ranged from 13-fold (for LIN) to 33-fold (for DHA). The decline in the value of n-3 PUFAs was more pronounced (25-fold) than that of n-6 PUFAs (15-fold). The river mouth area was characterized by a lower nutritional value of benthic macroinvertebrate community compared to the community in the lower reaches before the flood for LIN and ALA. The contents of ARA, EPA, DHA, n-3, and n-6 PUFAs did not differ significantly between these two sections (Figure 5).

3.5. Fatty Acid Analysis of Riverine and Marine Littoral Crabs

Factor 1, explaining 33.6% of the total variance, represented a gradient from fatty acids with negative scores (16:2n-6, ALA, i15:0, 16:3n-4, and 16:3n-3) to those with positive scores (ARA and DHA). Factor 2, explaining 24.7% of the total variance, represented a gradient from fatty acids with negative scores (17:0 and 20:2n-6) to those with positive scores (16:0, 14:0, and 18:4n-3) (Figure 6). Visually, marine littoral crabs were spatially separated from riverine crabs, with the marine littoral crabs forming a tighter cluster than those from the river (Figure 6). Indeed, marine littoral crabs had significantly higher percentages of marine algal FA-markers: DHA (6.6 ± 0.75 vs. 3.3 ± 0.58, $p = 0.0054$, $t(8) = -3.78$), ARA (5.7 ± 0.52 vs. 3.7 ± 0.54, $p = 0.016$, $t(8) = -3.05$), and 18:4n-3 (1.1 ± 0.15 vs. 0.3 ± 0.07, $p = 0.008$, $U = 0.0$). For 14:0, only a trend towards a higher level in marine littoral crabs was observed (2.2 ± 0.26 vs. 1.3 ± 0.16, $p = 0.056$, $U = 2.0$). In contrast, the levels of FA-markers of cyanobacteria, green and diatom algae, and aquatic macrophytes were significantly higher in riverine crabs: 16:3n-4 (1.0 ± 0.2 vs. 0.5 ± 0.03, $p = 0.008$, $U = 0.0$), LIN (8.9 ± 3.71 vs. 2.8 ± 0.08, $p = 0.008$, $U = 0.0$), and ALA (4.6 ± 1.21 vs. 2.0 ± 0.22, $p = 0.032$, $U = 1.0$). For

17:0 (0.9 ± 0.07 vs. 0.6 ± 0.06, $p = 0.056$, $U = 2.0$) and 16:3n-3 (0.6 ± 0.34 vs. 0.2 ± 0.02, $p = 0.056$, $U = 2.0$), only a trend towards higher levels in riverine crabs was detected.

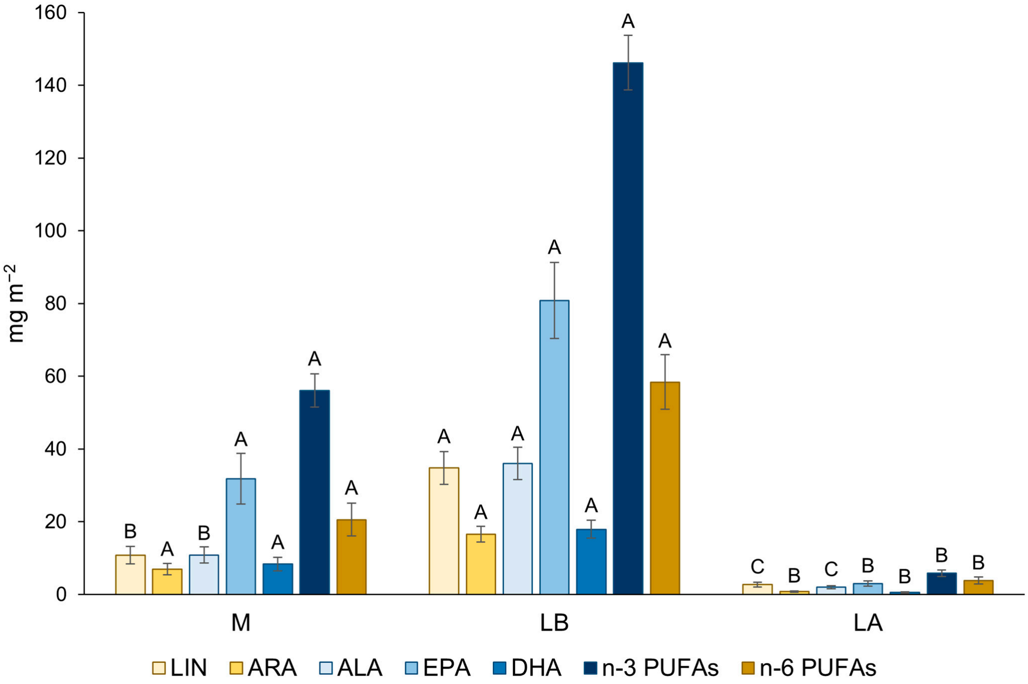

Figure 5. Content of essential and physiologically valuable fatty acids and the sum of n-3 and n-6 PUFAs (mg m^{-2}, M ± SE) before rainfall flood at the river mouth area (M), before the flood in the lower reaches (LB), and after the flood in the lower reaches (LA) of the Anastasia River, Sakhalin Island, August 2025. Mean values labelled with different letters differ significantly at $p < 0.05$ (Welch's ANOVA, Games–Howell post hoc test with correction for multiple comparisons, comparisons between sites for each PUFA). Fatty acid abbreviations: linoleic acid (LIN, 18:2n-6), arachidonic acid (ARA, 20:4n-6), eicosapentaenoic acid (EPA, 20:5n-3), and docosahexaenoic acid (DHA, 22:6n-3).

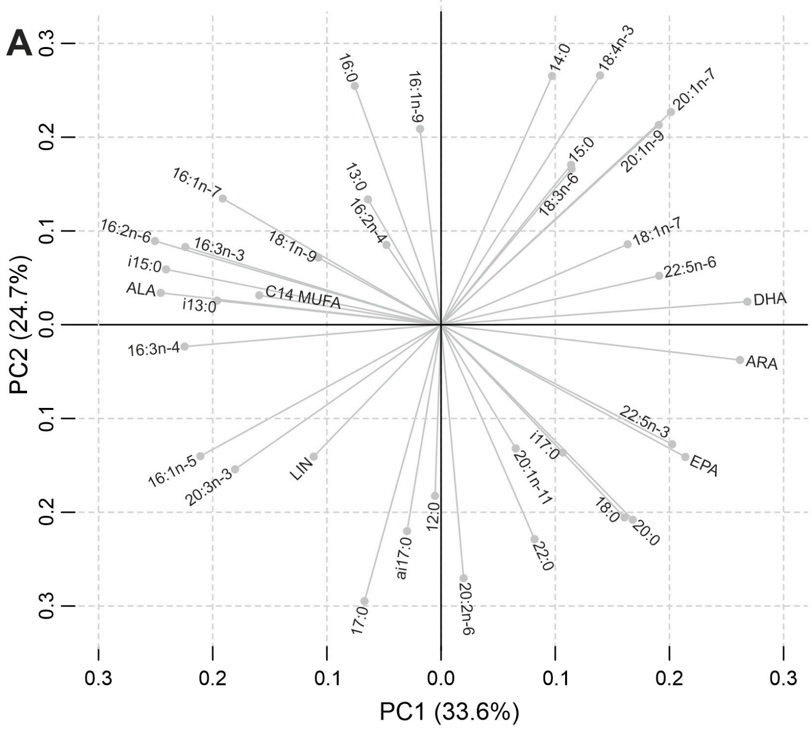

Figure 6. *Cont.*

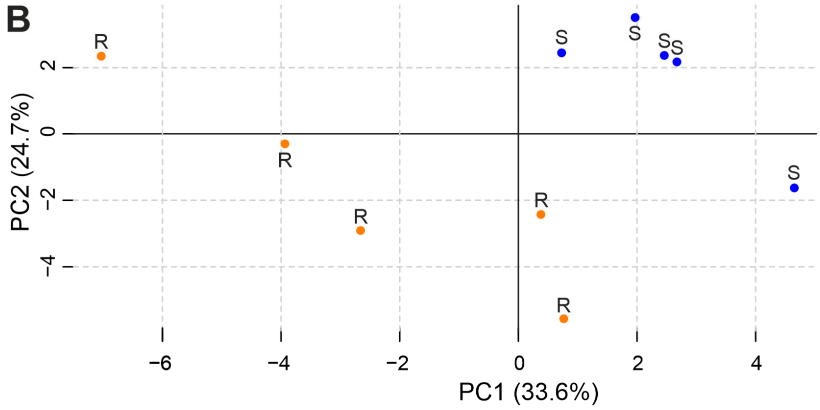

Figure 6. Principal component analysis (PCA) biplot visualizing the relative percentages of fatty acids (**A**) in crabs (*Eriocheir japonica*) from the Anastasia River and the marine littoral zone (**B**). PC1 and PC2 explain 33.6% and 24.7% of the total variance, respectively. Symbols: R (orange)—riverine crabs, S (blue)—marine littoral crabs. Fatty acid abbreviations: linoleic acid (LIN, 18:2n-6), arachidonic acid (ARA, 20:4n-6), eicosapentaenoic acid (EPA, 20:5n-3), and docosahexaenoic acid (DHA, 22:6n-3).

4. Discussion

Our study demonstrates that extreme rainfall events in southern Sakhalin lead not only to a structural change in the benthic macroinvertebrate communities, but also to a significant reduction in their nutritional value. For the first time, we have quantitatively shown that the losses of n-3 PUFAs exceed the losses of n-6 PUFAs by a factor of 1.7, decreasing 25-fold after the flood. Furthermore, the losses of the most valuable LC-PUFAs for fish (EPA, DHA, and ARA) were 1.8 times greater than the losses of shorter-chain PUFAs (LIN and ALA), precursors in synthesis of n-3 and n-6 LC-PUFAs. This implies that after a flood, fish will be limited not only by the quantity of food but also by its quality.

The amphipod *E. kygi*, rich in LC-PUFA (especially in DHA and ARA), was prey of higher nutritional value for fish in the Anastasia River. This amphipod is a mass-dominant and structure-forming component of benthic communities in some Sakhalin rivers [54]. Riverine amphipods can indeed be the most valuable source of lipids and, in particular, physiologically essential PUFAs for fish [8,55]. The significant drop in their biomass after the flood (from 97% to 64%) drove disproportionately high losses of n-3 PUFAs. Insects, whose proportion increased, do not compensate for this loss, as they do not synthesize or accumulate DHA and other C22 PUFAs. However, a disproportionate decline also affected EPA, the content of which in insects was as high as in amphipods. During the flood, in addition to the general drift of amphipods, there was also the elimination of medium-sized and, for some taxa, small-sized groups, which apparently had high nutritional value. In amphipods, young individuals were more susceptible to floods than large ones. We did not find any studies on size-dependent changes in fatty acid content in riverine macroinvertebrates, making this a promising topic for future research. A probable higher nutritional value of small- and medium-sized amphipods may be explained by several physiological and ecological mechanisms. First, ontogenetic dietary shifts are common in amphipods. Juveniles often feed on qualitatively different resources such as high-quality biofilms, bacteria, or specific algae compared to adults, which can result in age-specific fatty acid profiles [56]. Second, the nutritional value of the small-sized amphipod species *Paramoera* sp. may have been higher than that of the larger species *E. kygi*. Interspecific variation in fatty acid composition, including EPA and DHA content, was demonstrated in amphipods [45]. Thus, the flood may have selectively eliminated either juvenile stages of the dominant species or amphipod species characterized by high nutritional value. Our findings complement existing knowledge about the impact of severe floods on quantitative macroinvertebrate parameters, such as biomass and density [39–41,48,57], demonstrating that qualitative (biochemical) changes can be even more pronounced and critical for higher-order consumers.

This study was conducted on a single river following a single extreme flood event. Our findings strongly suggest that a decline in the proportion of amphipods, coupled with an increase in insect larvae within the community, leads to a reduction in C22 PUFA levels. The duration of the low biochemical quality of the benthic macroinvertebrate community after severe floods is likely linked to the ecosystem's recovery rate. The recovery of benthic communities is known to be a long-duration process, ranging from several weeks [40] to several months [42], and in some cases, reorganization can last for several years [58]. This process can be influenced by multiple factors, including post-flood changes in water quality (e.g., nutrients, suspended solids) [40,59]. The long-term consequences for the health status of fish such as salmonids can be substantial. A decrease in dietary n-3 LC-PUFA directly leads to reduced contents of these biomolecules in consumer tissues, including muscles, liver, heart, and fat [8,55]. A sustained lack of n-3 LC-PUFA in the fish diet results in a decline in fish health, leading to a range of pathological conditions (myocarditis, hepatic and intestinal steatosis, fin erosion, spinal deformities, etc.), a reduction in the population's growth rate, and an increase in fish mortality [24,25,60].

The use of FA-markers, whose application is extensively described in the literature [52,53], made it possible to determine the diet of the studied benthic macroinvertebrates and reveal their important ecological features. The high content of algae markers (ALA and C16 PUFAs), bacterial markers (branched FAs), and allochthonous OM (LIN) in amphipods confirms their role in the processing of terrestrial plant material and integrating various trophic pathways within the river ecosystem. Moreover, the increase in proportion of LIN in amphipods from 6% to 12% with distance from the estuary, indicates a greater contribution of allochthonous OM to the diet of these shredders. Diptera likely also participated in the consumption of allochthonous OM, as they contained the highest percentages of LIN. However, a cyanobacterial or fungal origin of this PUFA in Diptera cannot be ruled out, as it can be highly represented in some species or strains [61,62]. The highest degree of consumption of diverse algae (cyanobacteria, greens, diatoms, and cryptophytes) was found in caddisflies, especially in *Goera* sp. Other studied caddisflies consumed allochthonous OM, as a characteristic feature of their FA profile was high levels of 18:0, 20:0, and 22:0, which are markers of detritus and terrestrial plants [63,64]. Mayflies demonstrated feeding on autochthonous organisms, primarily cyanobacteria and green microalgae with high ALA content, and diatoms. Given that the most abundant species in the studied section of the Anastasia River was *Rhithrogena* gr. *lepnevae*, a grazer–scraper, our data on mayfly feeding preferences are consistent with those reported in the studies on periphyton consumption by mayflies [65,66]. The diet of stoneflies *Leuctra* sp. and *Sweltsa* sp. showed the highest proportion of detritus, including of allochthonous origin, and associated bacteria. The dietary preferences we identified for these stoneflies are consistent with previous studies based on gut content analysis of representatives of these genera [67]. However, some species of the genus *Sweltsa* are omnivorous and even predatory, as revealed by stable isotope analysis [68].

The differences found in the FA profile between freshwater and marine crabs *E. japonica* indicate significant trophic plasticity in this species and adaptation to available resources, which has not been previously described for Sakhalin populations. The river crabs were close in FA profile to amphipods, which reflects their phylogenetic relationship and demonstrates the similarity in the feeding spectra of these crustaceans.

Our results shift the problem of the impact of rainfall floods from the plane of "reduced food quantity" to the plane of "educed food quality". Given the predicted increase in the frequency and intensity of heavy rainfall in the Far East, the effect we have identified may become a regular factor limiting the productivity of river ecosystems on Sakhalin Island, which are key spawning and permanent habitats for many fish species. While some valuable commercial fish species (pink and chum salmon) migrate to the sea shortly after hatching, other salmonids, such as Sakhalin taimen, char, the whitespotted char, masu salmon, and coho salmon, spend several years in rivers feeding on invertebrates before shifting to piscivory [69]. Among these fish, Sakhalin taimen (*Parahucho perryi* Brevoort, 1856), which spends the first 2–7 years of its life in rivers, is listed as a priority species requiring urgent restoration and conservation measures [70,71]. The prolonged recovery of the nutritional value of benthic macroinvertebrates can create "trophic windows" of unfavorable conditions for the entire fish fauna, including valuable fish species.

5. Conclusions

The comprehensive study conducted, integrating taxonomic and biochemical approaches, has for the first time demonstrated that extreme rainfall floods in the south of Sakhalin lead to a profound degradation of not only the quantitative (biomass and density) but also the qualitative (fatty acid content) characteristics of the benthic macroinvertebrate communities. Dominant amphipods, which are the primary source of long-chain polyunsaturated fatty acids, were the most vulnerable to the disturbance, limiting the food resources for the fish.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/environments13040183/s1, Figure S1: The mouth of the Anastasia River before (A) and the day after the heavy rainfall (B); Table S1: The taxonomic composition of benthic macroinvertebrates in the Anastasia River, August 2025; Table S2: Major fatty acids (% of total fatty acids and mg g–1 wet weight, M ± SE) in dominant benthic macroinvertebrates in the lower reaches of the Anastasia River, Sakhalin Island, August 2025.

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Abbreviations

The following abbreviations are used in this manuscript:

FA	fatty acid
ALA	alpha-linolenic acid (18:3n-3)
LIN	linoleic acid (18:2n-6)
ARA	arachidonic acid (20:4n-6)
EPA	eicosapentaenoic acid (20:5n-3)
DHA	docosahexaenoic acid (22:6n-3)
SFA	saturated fatty acid
MUFA	monounsaturated fatty acid
PUFA	polyunsaturated fatty acid
LC-PUFA	long-chain polyunsaturated fatty acid
FAME	fatty acid methyl esters
OM	organic matter
PCA	principal component analysis

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