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Abstract: The green macroalga *Cladophora glomerata*, a species typical of brackish water, predominates in most coastal areas of estuarine ecosystems. The present study aimed to determine the current ecological conditions in the Neva estuary (Baltic Sea) when subjected to eutrophication and the summer *Cladophora* bloom. Macroalgae bloom can result in temporary unfavorable conditions (oxygen depletion and pollution) for invertebrates during macroalgae decomposition, and its contribution to the autochthonous benthic food web remains unclear. We evaluated the *Cladophora* biomass and the abundance and composition of macro- and meiobenthic invertebrates and traced trophic links in the coastal area of the Neva estuary during the *Cladophora* bloom. Some species of grazing or omnivorous consumers (nematodes, gastropods, amphipods, insect larvae) reached high abundance in the *Cladophora*-dominated coastal community. The tracing of food sources in a food chain of the *Cladophora*-dominated coastal community (macrophytes-grazers-omnivores) were elucidated using dual δ^{13} C and δ^{15} N stable isotope analysis. The results showed that autochthonous organic sources derived from *Cladophora* at various stages might contribute notably (up to 89%) to the coastal food web, supporting the production of benthic consumers.

Keywords: macroalgae; benthic invertebrates; coastal food web; trophic interactions



Globally, green algae (Chlorophyta), were found to be the most reported and widespread blooming macroalgae. The main factors promoting macroalgae blooms are an excess of nutrients caused by human activities, including agriculture, aquaculture, industrial, and domestic effluents, as well as an increase in average global temperatures, which has intensified blooms of macroalgae in estuaries in recent years [1]. Recent reviews have synthesized the evidence for the effects of ephemeral macroalgae blooms on the organization and dynamics of marine and estuarine ecosystems [2,3].

The largest (3600 km²) of the estuaries of the Baltic Sea, the Neva River estuary (Gulf of Finland), is affected by irregular marine water intrusions due to the stochastic water exchange with the Baltic Proper and is characterized by a horizontal gradient of water salinity from 0.1 to 7 g/L. Currently, active eutrophication is taking place in the Neva estuary. Intensive submerged vegetation responds to increased nutrient loading by shifting from slow-growing plants to fast-growing macroalgae and the ultimate dominance of phytoplankton at high nutrient levels [4]. This shift reflects a change from nutrients to light limitation of primary producers under eutrophication. In the Neva estuary, micro- and macroalgae blooms (mainly genera *Cladophora* and *Ulva*) are common summer phenomena. The chlorophyll *a* in water can reach 15 μ g/L [5]. The green macroalga *Cladophora glomerata*, typical of a brackish water complex of species [6,7], predominates in coastal areas of the Neva estuary [8]. The production of macroalga *Cladophora* together with associated epiphytes, achieved 7 g C/m² day (assuming 10% of carbon in the wet weight of macroalgae; [8]). According to [9], the total biomass of epiphytes (microalgae) on *Cladophora* achieved 32% and averaged 7.7% of the total algal tissue.



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In mid-summer, macroalgae reach high biomass (i.e., Cladophora bloom occurs) and, after storms and windy weather, massive floating/drifting mats and storm casts accumulate along the coastal zone and on the beach. Two main events cause an accumulation of algal biomass at the shoreline: wind transfer and the subsequent short-term variability of the sea level. Wind and surf tear off algae from the substrate and transport them to the shore, and if the water level decreases, huge masses of algae remain on the coast [10]. Macroalgal detritus concentrates sea garbage (macro- and microplastics) and pollutants (petroleum hydrocarbons and heavy metals [11–13]), thereby accelerating the transit of these substances from the sea to the coast and back. In addition, accumulated macroalgae in the coastal waters leads to a decrease in oxygen content in the water and sediment under the mats, forming anoxic conditions which favor the production of hydrogen sulfide in sediments [14]. The decomposition of *Cladophora* notably increases phosphorus concentrations in the overlying water and promotes the release of phosphorus from sediments into the overlying water [15]. Some species of the genus *Cladophora (C. rupestris)* contain a group of toxic metabolites of microscopic fungi, such as zearalenone and ergoalkaloids [16]. These phenomena provoke remobilization of adsorbed metals, nutrients, and other compounds in the coastal ecosystem [17].

Blooms of macroalgae and the formation of great masses of benthic organic matter in the coastal zone increase the respiratory demand for dissolved oxygen at night when photosynthesis is not occurring [18,19]. The oxygen depletion can alter zoobenthic communities and decline the abundance of herbivores [20]. Further problems arise when macroalgal blooms begin to decompose, as they can release harmful gases and metabolic byproducts such as ammonium [21], hydrogen sulfide [8], carbon disulfide and methyl sulfide [22], which can lead to the death of animals. The effects of macroalgae on the abundance and distribution of invertebrates in coastal habitats depend on the macroalgal biomass [23] and are not uniformly negative. Algal accumulations can increase habitat complexity, provide tolerant species with food or shelter, and enhance both dispersal and secondary production [24]. Joniver et al. [2] showed that macroalgae blooms have a negative influence on fish and mollusks. In contrast, annelids and arthropods showed a wide range of responses, suggesting that the response of species in these taxonomic groups is species-specific, i.e., some are tolerant to the effects of macroalgae blooms and others are not. For example, the widespread mortality of animals in the shallow areas of the Neva estuary during the maximum drifting of the algal biomass [8] resulted in shifts of the benthic community structure and at least a tenfold decline in the biomass of invertebrates. However, some tolerant groups of grazers (mainly arthropods) with opportunistic life strategies inhabit the coastal area subjected to Cladophora blooms and the temporal oxygen depletion induced by macroalgae decomposition.

The maximum effects of drifting macroalgae were recorded at near-shore sites (0–20 m from the shore) with high biomasses (300–450 g DW/m²), while at greater distances (>30 m from the shore), the biomass of drift decreased, oxygen was above 3 mg/L (normoxia), and the biomass of benthic macrofauna reached typical values, 20–30 g WW/m² [25]. Thus, *Cladophora* blooms led to reductions in coastal water quality and influenced the coastal food web [26]. Adapted invertebrates can be abundant in coastal habitats and play an essential role for macroalgae detritus decomposition.

The trophic relationships between potential grazers and coastal food sources in *Cladophora*-dominated habitats remains poorly studied. Moreover, some studies using isotopic analysis and food web modeling have shown that macroalgae and perennial macrophytes variously contribute as food sources to the coastal food web. *Cladophora* was distinguished as an important food source for only a few selective grazers [27]. The consumption on another macroalga, *Pylaiella littoralis*, by crustaceans *Gammarus* increased only as its photosynthetic activity decreased during algae decomposition. Seemingly, algae has a protective mechanism from grazing in the early stages of the life cycle (young and actively photosynthetic macroalgae) [28]. Tomczak et al. [29] proved that macroalgae and perennial macrophytes contribute to the coastal food web via the detritus food chain; grazers did

not consume them directly. In the Baltic Sea, a passive import of organic matter in detritus form made up 20% of the total planktonic production in the Curonian lagoon [30]. In some areas (Gulf of Riga, Puck Bay), annual macrophytes positively influenced the biomass of benthos or fish [29].

This study focuses on the present ecological conditions of the Neva estuary coastal area, which is subjected to eutrophication and the summer *Cladophora* bloom. The main question of the work was to determine whether there is a positive contribution from the mass development of *Cladophora* for the coastal community, since in previous studies we did not find a clear answer to whether this autochthonous source of organic matter is used by local consumers. Otherwise, the effect of macroalgal blooms can only have a negative impact through the deterioration of physico-chemical conditions, leading to a low biomass of coastal inhabitants, which is confirmed by numerous studies in various estuaries. The macroalgae biomass, and the composition and abundance of macro- and meiobenthic invertebrates and their trophic relationships in the coastal community were investigated along the coastal line of the Neva estuary during the summer macroalgae bloom. The study aimed to estimate the contribution of macrophytes as primary sources to the autochthonous benthic food web. The diet composition of littoral consumers (grazers, detritivores and omnivores) and the predicted contribution of the dominating macroalga *C. glomerata, Ulva intestinalis* and perennial macrophyte *Potamogeton* spp. to the consumers' diets were elucidated using dual (δ^{13} C and δ^{15} N) stable isotope analysis (SIA).

2. Materials and Methods

2.1. Study Sites and Sample Collection

The Neva River forms one of the largest estuaries in the Baltic Sea. The freshwater part of Neva Bay (400 km²) is fenced off from the open bay by the protective dam of St. Petersburg. Ten sites in the Neva estuary coastal zone were surveyed in July 2021. This is a period of active proliferation of *Cladophora* and other aquatic plants in the coastal zone (Supplementary Figure S1). Depth at study sites varied from 0.4 to 0.7 m; gravel, stones, coarse sand, and detritus covered the bottom. The macroalgae, mainly *Cladophora glomerata*, grew on hard substrata and belts of *Potamogeton* spp., with epiphytic microalgae developed on soft sediment. At some locations *Cladophora* belts coexist with *Ulva* beds.

Salinity, temperature, and oxygen were measured in the surface and near-bottom water using the Conductivity meter (HANNA DistWP4) and Oxygen meter (HANNA HI9142) on site. The concentration of total phosphorus in the water was determined by photometry. Water samples (0.25 mL) for the detection of phosphorus were collected in clean bottles with several drops of H_2SO_4 and analyzed in the Lab according to standard techniques [31]. Biotesting for crustacean survival remains a very effective method for assessing the environmental quality of habitats. The 10-day survival tests with amphipod *Gmelinoides fasciatus* were used for the quality assessment of sediments. The sediments (three cm of the upper layer) were collected at each study site by bottom grab (Ekman), and amphipods *G. fasciatus* were collected from a clean location. The content of organic carbon in sediments was measured as a percentage of the dry mass of bottom sediments using an analyzer AN-7529M (Gomel, Belarus).

Macroalgae and invertebrates were collected in coastal sites (approximately 20 m from the shoreline) from 0.4 to 0.7 m. The growing macroalgae and benthic macroinvertebrates associated with macroalgae were sampled using a cylindrical metal frame (a 0.8 m height and a sampling area of 0.03 m^2) and a hand net in three replications per site to evaluate their biomass. The algae attached to hard substances were collected from the frame, detached of substrates, and washed with fresh water to remove all animals. The algae were air-dried to a constant weight in the laboratory and weighed to 0.01 g precision. Their biomass was estimated as an arithmetic mean of dry weight (DW) \pm SE (standard error) per 1 m² of the bottom area.

Procedure of macrozoobenthos collection was proposed previously [25]. In short, the 0.03 tube frame was forced into the bottom and all the hard substrates (stones) and

plants in the frame were transferred to fresh water in a plastic container. Animals were washed off plants and stones; attached mollusks were scraped off with a knife). In addition, 3-7 cm layer of soft sediments (after stones were removed) and animals from the water column were collected with a hand net of 0.5 mm mesh size. All parts of the sample were combined in the plastic container, washed with water, sieved (0.5 mm), and then conserved with 4%-formaldehyde in plastic zip-bags. Meiobenthic samples were collected by a tube sampler (sampling area 20 cm²) to a 5 cm depth in three replicates at each site and preserved with a 10%-formaldehyde in glass bottles and processed under a microscope in a laboratory. The identification of invertebrate species was carried out by specialists in the taxonomy of the nematodes (by V.A. Petukhov), oligochaetes (I.G. Tsyplenkina), and macrobenthos (N.A. Berezina).

To trace the autochthonous food web, all dominated groups of invertebrates, small fish (Gobiidae), growing macroalgae, drifting filaments (drift), and algae-derived detritus were collected at sites 2 and 4 manually or with a hand net. These samples were transported in natural water in an isothermal box to the laboratory to be prepared for SIA. We have chosen these two locations as representative of the typical types of communities found in parts of the Gulf of Finland, that is, sites with widespread and numerous species of model consumers, which are also found in other areas. It was impossible to examine more cases of communities due to the complexity of sampling all the links, the logistics failure of delivering the consumers in a live form to the laboratory, and the rather long time needed for further laboratory preparation of the collected materials for SIA.

2.2. Environmental State

The quality of the sediments was assessed in a 10-day bioassay test following grading criteria based on the survival rate (%) of amphipod *G. fasciatus* (AS), with the quality of sediments regarded as "very good" (1st class) at 90–100%, "good" (2nd class) at 70–89%, "satisfactory" (3rd class) at 50–69%, "poor" (4th class) at 20–50%, and "bad" (5th class) at <20% survival rate [32,33]. The two upper classes correspond to a good environmental state (GES), class 3 is considered as transitional from good to bad (sub-GES), and classes 4 and 5 are considered unfavorable conditions for fauna, i.e., a bad environmental state (BES).

2.3. Stableisotope Analysis of the Coastal Food Web

Ranging from 100 to 150 mg of growing aquatic plants (*Cladophora, Ulva,* and *Potamogeton*), drifting filamentous algae, and algae-derived detritus were used for the sample preparations. Samples were rinsed with distilled water and cut into small pieces of approximately 9 mm² for further drying. Collected animals were held in clean water for 12 h for the evacuation of their gut and then rinsed with distilled water and dissected (if applicable) before drying.

Muscle tissues in the case of mollusks, legs of arthropods, head parts of annelids (hirudineans, oligochaetes), and fish muscle tissue were collected from three to five specimens belonging to each species. Whole bodies of small organisms, such as larvae of chironomids, *Agraylea*, and *Caenis* were taken in five replicates.

Plants, detritus, and animals were dried for 48–72 h at 55 °C. After drying, a homogeneous powder of each sample was prepared using a pestle and mortar.

The food web structure was assessed using carbon (C) and nitrogen (N) stable isotope composition. SIA was performed in the joint usage center at the A.N. Severtsov Institute of Ecology and Evolution RAS (Moscow, Russia) using an isotope ratio mass spectrometer Thermo Finnigan Delta V Plus connecting to an elemental analyzer (Thermo Scientific Flash EA 1112, Delft, The Netherlands). Approximately 0.3–0.5 mg of powder of animal organisms and 0.9–1.2 mg of plants/detritus were put into tin capsules and weighed using a Mettler Toledo MX 5 (Mettler, Columbus, OH, USA) balance with a precision of $\pm 1 \mu g$. The isotopic composition was expressed in δ units based on the relative difference (in parts per thousand) between the sample and international standards according to the equation:

 $\delta R(\infty) = (R \text{ sample}/R \text{ standard} - 1) \times 1000$, where R is the molar ratio of heavy/light isotopes, i.e., ${}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$. δR is the relative deviation (for example, $\delta^{13}C$ or $\delta^{15}N$) of the sample from the generally accepted international standard (for carbon, the Vienna equivalent of fossil belemnite from the PeeDee Formation, VPDB; for nitrogen, atmospheric N₂). The signs "+" and "-" will indicate a greater or lesser content of the heavy isotope compared to that of the standard.

The stable isotope signatures were used to estimate the contribution of prey (sources) to the diet of the consumers. With three or more possible food sources and the availability of isotope data for only two elements (C and N), two main strategies are used combining sources and mixing modeling. Modern mixing models (IsoSource [34], MixSIR [35], SIAR [36], MixSIAR [37]) provide integration of the observed variability in source and mixture tracer signatures. To calculate the contribution of various food resources to the diet of consumers grazing on macroalgae (gastropods, ephemeropterans, amphipods) and omnivorous consumers (hirudineans and fish), we used MixSIAR package from open-source R package (CRAN, GitHub, [37]). Four food sources, including two species of macroalgae *Cladophora* (together drift and attached) and *Ulva*, perennial macrophytes (*Potamogeton*), algae-derived detritus, and various groups of consumers were considered in mixing modeling basing on data from site 2, from which the most detailed data were obtained. The trophic enrichment in ¹³C and ¹⁵N in the modeling of diets was accepted as 1.3 ± 0.6‰ and 3.4 ± 1.0‰, respectively.

2.4. Statistics

All variables were expressed as mean value and standard deviation for SIA data and standard error for biomass and environmental parameters. The non-parametric Spearman correlation was used to determine correlations between site characteristics, benthic, and macroalgae biomass. The significance of variables' differences between sites was tested using the Kruskal–Wallis test (non-parametric ANOVA), followed by a Mann–Whitney pairwise comparisons test. Significant differences between variables were accepted at p < 0.05. All calculations were performed using STATISTICA 10.0 software (https://statistica.software.informer.com/; accessed on 10 September 2022).

3. Results

Table 1 summarizes data on the total dissolved salt content and phosphorus concentration in the water. The low salt content (0.1–0.5 mg/L) was recorded at sites 4–6 (Neva Bay) due to the strong influence of the freshwater of the influent Neva River. Water salinity increased considerably outside the dam in the inner estuary (Figure 1), mixing with mesohaline waters from the open Gulf of Finland. At site 10, the salinity reached a maximum of 3 g/L. Total phosphorus concentration ranged from 42 to 148 mkg/L, with the highest value at site 8 (Grafskaya Bay) on the southern coast of the Neva estuary. All the studied sites ranged within values that characterized the "eutrophic conditions" with nutrient-rich water. The environmental state based on the amphipod survival test was good at five sites (Table 1). We revealed the only case (site 8) with bad environmental conditions. The survival rate of the tested amphipods correlated negatively with the concentration of total phosphorus in the water (Spearman, *R* = 0.80, *p* < 0.01, Supplementary Table S1), obviously testifying to the level of eutrophication at the study sites.

3.1. Macroalgae

The biomass of macroalgae varied significantly between sites (Figure 2). At site 2, two species of macroalgae were important, *C. glomerata* and *U. intestinalis*. The highest biomass of *Cladophora* was at site 2 (96.8 g/m² in dry weight, DW) and the lowest at site 9 (16.8 g DW/m², Figure 2). At other sites, only *C. glomerata*, among ephemeral macroalgae, was recorded. The level of *Cladophora* biomass correlated positively with water salinity (Spearman, R = 0.63, p < 0.05) and negatively with the level of organic carbon in the sediments (R = -0.70, p < 0.05). At the typically freshwater sites 4–7, the attached

Cladophora was practically not found. However, drifting algae mats were present at site 4. The biomass of drifting algae at site 2 and site 4 were estimated as 101 and 130 g DW/m², which is close to the lowest biomass of drifting belts recorded earlier in the estuary and other parts of the sea [8,28].

Table 1. Study sites' location, physical, and chemical characteristics. P is phosphorus and C is carbon in sediment, AS is amphipod survival, GES and BES mean good and bad environmental state.

Site	Name	N; E	Salinity, g/L		Oxygen, mg/L		P Total,	Carls at mate	C arra %		<u></u>
			Surface	Bottom	Surface	Bottom	mkg/L	Substrate	C UIg /	AS %	State
1	Primorsk	60.36; 28.61	2.1	2.2	7.2	7.1	125	Silty sand, stones	0.4	60	Sub-GES
2	Flotsky	60.16; 29.16	1.5	1.6	7.1	7.0	60	The same	0.3	94	GES
3	Serovo	60.20; 29.57	1.0	1.1	7.2	7.1	50	The same	0.5	90	GES
4	Repino	60.16; 29.85	0.5	0.6	7.2	7.1	102	The same	0.5	70	Sub-GES
5	Olgino	59.99; 30.10	0.1	0.2	7.0	7.0	66	The same	0.5	55	Sub-GES
6	Petergof	59.89; 29.90	0.1	0.2	7.2	7.0	88	The same	0.5	65	Sub-GES
7	B. Izhora	59.96; 29.50	1.6	1.7	7.3	7.2	45	Sand	0.1	100	GES
8	Grafskaya	59.98; 29.20	2.2	2.3	7.3	7.2	148	Silty Sand	0.5	40	BES
9	Sista-Palkino	59.81; 28.91	2.0	2.1	7.3	7.2	50	Silty Sand	0.5	75	GES
10	Luga Bay	59.83; 28.50	2.8	2.9	7.3	7.2	42	Sand, stones	0.1	90	GES



Figure 1. Map showing location of ten study sites in the Neva estuary (Baltic Sea). Characteristics of these sites are given in Table 1.

3.2. Benthic Community

Altogether above 70 taxa of benthic animals were identified at the study sites (Supplementary Table S2). Nematodes, oligochaetes, and chironomids were the richest groups in species number. Meiofauna was abundant at sites 2 and 3 because of the great contribution (69–71%) of small-sized oligochaetes and chironomids. The biomass and abundance of meiobenthos were very low at sites 6, 7, and 10 (Figure 3). Five species of nematodes *Epitobrilus medius*, *Brevitobrilus stephanskii*, *Raritobrilus steineri*, *Tobrilus gracilis*, and *Mononchus truncates* were abundant taxa at all sites.



Figure 2. Mean biomass in dry weight (g/m^2) and standard error (+1SE, n = 3) of growing *Cladophora glomerata* at study sites.



Figure 3. Means + 1SE (n = 3) of abundance, thousands ind/m⁻² (**a**) and biomass (**b**) in wet weight, g/m^2 of meiobenthos at study sites.

Invertebrate grazers were represented mainly by crustaceans (amphipods *Gammarus tigrinus*, *G. zaddachi*, *Gmelinoides fasciatus*, *Pontogammarus robustoides*, isopod *Asellus aquaticus*), gastropods (mainly *Theodoxus fluviatilis*, *Bythinia tentaculata*, *Radix balthica*), and various species of Chironomidae, Trichoptera, and Ephemeroptera (Supplementary Tables S2 and S3). The greatest biomass of macrofauna was found at site 1 (25 g WW/m²) and site 10 (29 g WW/m²).

Figure 4a); both sites are characterized by the high contribution of mollusks and crustaceans (amphipods). A significant positive correlation was revealed between biomasses macrozoobenthos and *Cladophora* (Spearman, R = 0.79, p < 0.05). In addition, the biomass of macrobenthic animals correlated positively with water salinity (R = 0.63), and negatively with sediment organic carbon (R = -0.70, p < 0.05).



Figure 4. Biomass of macrozoobenthos g WW/m², mean \pm 1SE, n = 3 (**a**) and percentage contribution of the most abundant taxa to the total biomass (**b**) at study sites.

Gastropod mollusks, amphipods, and aquatic insects' larvae (Ephemeroptera, Trichoptera) made the largest contributions to the biomass of the benthic community (Figure 4b). Species of several feeding groups dominated among the consumers: shredders (insect larvae), grazer-scrapers (gastropods), and collector-gatherers (crustaceans, Supplementary Table S2). The gastropod *Theodoxus* is known to be an efficient consumer of diatoms and microalgae living on macroalgae filaments (epiphytes) [38]. Some species of gammaridean amphipods (*Gammarus* spp., *P. robustoides*) belong to consumers with a mixed food strategy (omnivores), and they are potentially able to consume filamentous macroalgae/plant material (acting as grazers), fine detritus (acting as detritivores), and other invertebrates (acting as predator). Nematodes may consume many objects, including bacteria, cyanobacteria, green, diatom microalgae, detritus, and dissolved organic matter [39]. Some species of Nematoda can act as an invertebrate predator, consuming ciliates, other nematodes, and oligochaetes (Supplementary Table S2).

The values of δ^{13} C and δ^{15} N of primary sources and the main members of the coastal food web are given in Table 2. The Isotopic compositions of consumers were different between various species (Kruskal–Wallis, Mann–Whitney tests in the case of p < 0.05, Supplementary Table S4). Differences in δ^{13} C values between sites were significant for the same taxa of consumers (H = 16.7, p = 0.02) while differences in δ^{15} N values were

insignificant (H = 0.46, p > 0.05). The mean δ^{13} C value for attached *Cladophora* at site 2 was 15.8‰ and 12.6‰ at site 4. At both sites, attached and drifting macroalgae differed little in the isotopic composition (H = 0.46, p > 0.05). The isotopic signature of detritus was similar at two sites and at site 2 differed considerably (H = 8.69, p = 0.03) from that of alive forms of macroalgae (Table 2). The δ^{15} N value of *Ulva* were significantly higher (*Mann–Whitney test*, p = 0.003) than the δ^{15} N value of *Cladophora* (Table 2). Figure 5 showed stable isotopes signatures of various forms of macroalgae, other plants, and algae-derived detritus and their potential grazers. The closest values of isotopic markers in the coastal community at site 2 were determined between *Cladophora* (attached and drifting) and the gastropod *Theodoxus*, and at site 4 between *Cladophora* and the trichopteran *Agraylea*.

Table 2. Isotopic composition of carbon and nitrogen (δ^{13} C and δ^{15} N values), total carbon (C), and nitrogen (N) content and mass ratio of C/N of the members of coastal food webs at sites 2 and 4. n = 5 for primary producers and detritus, n = 4 for consumers.

		Site 2					Site 4					
Group/Variable		δ ¹³ C	$\delta^{15}N$	%C	%N	C/N	$\delta^{13}C$	$\delta^{15}N$	%C	%N	C/N	
Seston	Mean	-22.9	4.8	-	-	-	-	-	-	-	-	
	SD	1.7	0.6	-	-	-	-	-	-	-	-	
Cladophora	Mean	-15.8	7.1	40.7	3.3	12.2	-12.6	5.8	35.8	3.8	9.5	
	SD	0.6	0.1	0.7	0.1	0.1	0.2	0.2	1.0	0.1	0.2	
Drifting mats	mean	-15.6	7.2	36.0	3.0	12.0	-11.5	6.8	35.2	3.3	10.6	
-	SD	0.5	0.1	1.1	0.1	0.0	0.4	0.1	0.1	0.2	0.6	
Algae-derived detritus	mean	-10.9	6.3	38.3	2.9	13.3	-10.8	6.3	38.8	2.9	13.5	
-	SD	0.1	0.1	0.5	0.1	0.1	0.0	0.1	1.3	0.1	0.1	
Ulva	mean	-12.4	9.4	36.1	3.4	10.7	-	-	-	-	-	
	SD	0.4	0.5	6.1	0.4	0.8	-	-	-	-	-	
Potamogeton	mean	-16.4	8.0	41.4	3.7	11.4	-14.5	9.0	41.9	3.4	12.3	
-	SD	0.7	0.5	5.4	0.4	1.4	0.1	0.8	4.4	0.3	1.8	
Bivalvia	mean	-21.6	7.9	49.4	11.5	4.3	-22.9	7.8	45.0	10.5	4.3	
	SD	0.2	0.6	1.6	0.3	0.2	0.7	0.9	8.5	2.3	0.5	
Gastropoda	mean	-14.7	7.1	30.7	8.8	3.5	-21.3	5.3	37.9	8.3	4.6	
-	SD	1.4	0.1	1.6	0.9	0.5	0.5	0.3	6.7	1.9	0.3	
Trichoptera	mean	-19.3	9.3	49.2	9.8	5.1	-14.8	6.8	43.8	8.9	4.9	
-	SD	0.6	0.1	2.3	1.2	0.7	0.4	0.2	0.1	0.5	0.3	
Diptera	mean	-17.9	9.1	47.3	11.2	4.2	-18.4	8.5	47.4	9.4	5.1	
-	SD	0.3	0.0	0.3	0.3	0.1	0.0	0.3	0.1	0.5	0.2	
Ephemeroptera	mean	-17.4	9.2	47.5	10.3	4.6	-18.8	9.3	45.4	10.8	4.2	
	SD	0.7	0.6	3.5	0.4	0.2	0.6	0.4	3.6	1.4	0.3	
Hirudinea	mean	-16.8	11.0	50.3	11.2	4.5	-19.5	12.2	39.3	9.7	4.1	
	SD	0.4	0.4	1.2	0.4	0.3	0.0	0.0	5.6	1.3	0.0	
Gammaridae	mean	-16.6	8.7	36.9	8.2	4.5	-18.7	9.1	27.1	5.7	4.7	
	SD	0.7	0.2	2.5	0.6	0.3	0.8	0.4	6.7	1.2	0.3	
Fish	mean	-17.5	12.1	41.3	11.4	3.6	-20.5	14.1	42.0	12.5	3.4	
	SD	0.0	0.4	4.3	1.8	0.2	0.2	0.2	2.7	1.1	0.1	

Mixing modelling was applied for the coastal food web at site 2. Modelling suggested close trophic relationships between *Cladophora* and representatives of Ephemeroptera and Gastropoda. Gastropods could receive at least 46% of the energy from the organic matter supplied by *Cladophora* and 26% by *Potamogeton* (Table 3). The potentially high contribution of *Cladophora* (up to 89%) as a food source was predicted for ephemeropteran larvae. The contribution of *Cladophora* to the diet of amphipods could achieve 46%, however the other source, *Potamogeton*, was the most important in their diet (up to 96%).

Ulva macroalga and macroalgal detritus were assessed as a minor part of the diet of gastropods and ephemeropterans but could reach up to 18% in the amphipod diet (Table 3). Algae-derived detritus contributed insignificantly to the diet of amphipods but more so to the diet of gastropods and ephemeropterans (up to 26 and 15%, respectively).



▲ St 2 • St 4

Figure 5. Stable isotopes signatures (‰) of various forms of macrophytes, algae-derived detritus and their potential grazers at site 2 (triangle) and site 4 (dots). Full data are given in Table 2. Green markers showed signatures of sources and black ones for consumers. Abbreviations: Pot.—*Potamogeton,* Clad.—*Cladophora*, Gam.—*Gammarus*, Chir.—Chironomidae, Hept. –*Heptagenia*. Erp.—*Erpobdella*.

Table 3. Predicted 2.5–97.5% quantiles and proportion mean \pm 1SD values of coastal producers' contributions to the diet of primary consumers and omnivores from the second trophic level (ephemeropteran *Heptagenia sulphurea* (*Eph*), amphipod *Gammarus tigrinus* (*Gam*) and gastropod *Theodoxus fluviatilis* (*Theo*) at site 2 based on Bayesian mixing model performed with MixSIAR package in R.

Sources	Eph		Gan	1	Theo		
Cladophora	< 0.01-0.89	0.36	< 0.01-0.46	0.16	0.46-0.48	0.47	
Potamogeton	< 0.01-0.95	0.54	0.42-0.96	0.74	0.26-0.28	0.27	
Ulva	< 0.01-0.2	0.06	< 0.01-0.18	0.06	< 0.001-0.01	< 0.001	
Detritus	< 0.01-0.15	0.04	< 0.01-0.03	0.03	0.25-0.26	0.26	

The consumers of the third trophic level of the coastal food web, hirudineans and fish, were supported by the organic matter of primary consumers. Mixing modelling also predicted that the amphipods are the basic energy resource contributors for omnivore consumers, such as hirudineans and gobiid fish, reaching 44 and 58%, respectively.

4. Discussion

The ecological status of most of the studied sites is quite similar. They were characterized by a high level of eutrophication (P total >50 mkg/L at most studied sites, except for sites 7 and 10). The results of biotesting identified the most polluted areas according to the state of the bottom sediments; they were closely dependent on the concentration of phosphorus in the water. The worst environmental state was assessed as site 8 (Grafskaya bay), where the calm conditions of the bay protected from the wind led to a slow water exchange and the highest level of the accumulation of nutrients and other substances. In such eutrophic conditions, the development of macroalgae is not limited by the level of nutrients, in particular phosphorus. The highest biomass of *Cladophora* was noted in the open part of the gulf (outer estuary, sites 1, 2, 8, 9, and 10) rather than in sites located within the Neva Bay (a shallow semi-enclosed lagoon) and the inner estuary, as these were more influenced by the fresh waters of the Neva River. Apparently, conditions for macroalgae are most favorable in the outer estuary due to a better water exchange and higher water salinity (macroalgae biomass positively correlates with water salinity). At the same time, the invertebrate biomass correlated with both factors.

Eutrophication and the phenomena of the macroalgae blooms in the Neva River estuary determine the structure of community in shallow water habitats, reducing their species richness and abundance [8,40]. This study and previous research in this area mboxciteB25-diversity-2027189,B40-diversity-2027189 showed that benthic community includes opportunistic taxa (r-strategists as a rule). That is, these are species with a short life cycle, rapid reproduction, and a wide range of nutrition. Amphipods (*P. robustoides*, *Gammarus* spp.) can avoid temporary adverse conditions (hypoxia, nutrient pollution, and other unfavorable factors) due to their ability to migrate to other areas with more favorable conditions. The positive correlation between the biomass of macrofauna and algae confirmed the close trophic relationship between these components of the coastal community. This feature was emphasized when studying the patterns of succession in coastal communities, when changes in the composition of animal communities and their trophic structure are usually closely related to the diversity of macroalgae or plants, their biomass [41], and the stage of their succession [42]. No correlation with aquatic plants was found for meiobenthos, which seems to be more related to the structure of bottom sediments and the amount of detritus and organic matter in them; these factors were not assessed in this work and require further study. The trend towards such an explanation is evident from the data obtained that the lowest meiobenthos indices were also found on sandy soils depleted in organic matter (sites 6, 10) and in the absence of macroalgal detritus (sites 6, 7).

Effects of grazing on macroalgae biomass were discussed broadly, but opinions are often contradictory. It was demonstrated that the grazing effects of amphipods (*Ampithoe longimana*) on macroalgal biomass might be notable only at an early stage of algal growing [43–45]. Late-stage decomposing macroalgae can lead to a sharp decrease in the benthic biomass [28].

An animal organism's isotopic signatures (δ^{15} N and δ^{13} C) generally reflect that of their diet with some offset due to trophic fractionation. They indicate the integrated composition of diets at different trophic levels over time [46,47]. The isotopic difference in carbon isotopic ratios between a consumer and its diet in the case of Cladophora was wider $(>2\infty)$ than is usually assumed (i.e., $0.1-1\infty$, [48]). The isotopic signature in sea grasses may vary locally in response to environmental factors such as light gradient and seasonal succession of plants [49]. The difference in nitrogen stable isotope ratios was within the widely accepted range (3–4 %, [48]). Unlike δ^{15} N values, the pronounced variability in δ^{13} C was found of macroalgae in freshwater and marine environments in other studies [39,50]. Marine macroalgae are characterized by δ^{13} C values varying widely from -11% to -20% [51,52]. The δ^{13} C values for seagrasses range from -23% to -3%, and values of the order of -10% are commonly observed [49,53]. For marine benthic microalgae, most values fall from -11% to -20% [54]. The average value of δ^{13} C for green algae is 15.6‰ [55], which is within the values obtained in this study for *Cladophora*. This value was close to the δ^{13} C value of amphipods, gastropods, ephemeropterans, and trichopterans. However, the isotope ratio of *Cladophora* can be changeable depending on the sources of dissolved carbon dioxide and bicarbonate, the growth rate, and environmental conditions [56], leading to spatial differences in δ^{13} C values detected in this study (from -11.5 to -15.8%).

Some decrease in the nitrogen content occurred in drifting algae (first decomposing stage), making this food source more valuable for primary consumers. The C/N ratio was significantly higher during macroalgae drifting and degradation (site 2), probably

due to their epiphytes and bacteria colonization. Changes in nutrient content are typical for various algae during decomposition and algae-derived detritus and act as a major queue [57–59]. Choo et al. (2017) [60] revealed that dense *Cladophora* belts with high photosynthetic rates can directly uptake organic carbon from the surrounding water in macroalgae tissue. In addition, the decomposing macroalgae can substantially modify nutrient dynamics in the water column and sediments, releasing large amounts of tissue phosphorus [61] and supporting the intensive growth of epiphytes on algae tissue [62]. Stable isotopes and source contribution to the diet analyses indicated that growing and drifting macroalgae and Potamogeton with epiphytes were essential food sources for gastropods, amphipods, and insects. At the same time, the number of species associated with the drifting belts (at various stages of degradation) was smaller compared with the green attached stage of Cladophora [25]. This is likely because the immobile species of grazers (mollusks and insect larvae) cannot directly colonize drifting algae, while selective mobile omnivores (amphipods), which could change their location, dominate in the drifting algae belts. Meiobenthic organisms, mainly nematodes, could colonize algal detritus and underlying sediments, as they are more resistant to low oxygen than macrofauna.

A comparison of *C. glomerata*'s and *U. intestinalis*'s nutritive qualities for potential grazers by fatty acid profiles, the contents of essential polyunsaturated fatty acids (PUFAs), and elemental composition showed a content ten times higher of eicosapentaenoic acid (EPA; 20:5n–3) in *Cladophora* biomass than in *Ulva* [63]. Thus, *C. glomerata* appeared to be a more valuable food for potential invertebrate consumers.

According to the recent results of mixing source modeling in the Gulf of Finland, benthic macroinvertebrates and fish mostly rely on pelagic-derived carbon as a basal resource for their production [27]. The primary production of *C. glomerata* varied from 3.6 to 7.9 g C/m² contributing to around 90% of the total primary production in the coastal ecosystem [8]. This study showed that macroalgae *Cladophora* and other coastal macrophytes might also contribute notably to energy sources for the coastal food web in the Neva estuary. The autochthonous *Cladophora*-derived energy sources support mostly littoral invertebrates that use grazing as a food strategy such as gastropods, insect larvae, and benthic omnivorous invertebrates. Despite the noticeable damage to coastal inhabitants in the estuary caused by decomposing macroalgae, including the mass death of organisms due to oxygen deterioration and coastal pollution, macroalgae have shown themselves to be an important source of organic matter that largely feeds the benthic community, increases the productivity of the coastal community, and maintains the food web of the entire ecosystem.

5. Conclusions

The excessive growth of opportunistic macroalgae, called macroalgal blooms, has become a widespread phenomenon in different parts of the Baltic Sea, constituting a significant nuisance to shallow coastal zones. *Cladophora* blooms create specific conditions in the coastal habitats leading to changes in the structure of invertebrate communities when several adapted groups can inhabit locations with a high development of macroalgae. This study showed that growing and drifting macroalgae formed essential food sources for coastal grazers, including gastropods, amphipods, and insect larvae, and formed the basic autochthonous food source for the coastal food web.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d14121053/s1, Table S1: Spearman correlation coefficient between variables of biota and environmental factors. Table S2: List of benthic species and functional groups at study sites. Table S3: Biomass of various groups of benthic invertebrates g WW/ m² and the total biomass of meio- and macrobenthos at study sites. Table S4: *p*-values after Mann-Whitney pairwise comparisons for consumers, Bonferroni corrected. Figure S1: Coastal zone of the Neva estuary during macroalgae bloom.

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References

- 1. Perrot, T.; Rossi, N.; Ménesguen, A.; Dumas, F. Modelling green macroalgal blooms on the coasts of Brittany, France to enhance water quality management. *J. Mar. Syst.* 2014, 132, 38–53. [CrossRef]
- 2. Joniver, C.F.H.; Photiades, A.; Moore, P.J.; Winters, A.L.; Woolmer, A.; Adams, J.M.M. The global problem of nuisance macroalgal blooms and pathways to its use in the circular economy. *Algal Res.* **2021**, *58*, 102407. [CrossRef]
- 3. Lyons, D.A.; Mant, R.C.; Bulleri, F.; Kotta, J.; Rilov, G.; Crowe, T.P. What are the effects of macroalgal blooms on the structure and functioning of marine ecosystems? A systematic review protocol. *Environ. Evid.* **2012**, *1*, 7. [CrossRef]
- 4. Duarte, C.M. Submerged aquatic vegetation in relation to different nutrient regimes. Ophelia 1995, 41, 87–112. [CrossRef]
- 5. Golubkov, M.S. Phytoplankton primary production in the Neva Estuary at the turn of the 21st century. *Inland Water Biol.* 2009, 2, 312–318. [CrossRef]
- 6. Gubelit, Y.I.; Kovalchuk, N.A. Macroalgal blooms and species diversity in the Transition Zone of the eastern Gulf of Finland. *Hydrobiologia* **2010**, *656*, 83–86. [CrossRef]
- Stepanyan, O.V. Distribution of macroalgae and sea grasses of the Sea of Azov, the Kerch Strait and the Taman Bay. *Oceanology* 2009, 49, 393–399.
- 8. Gubelit, Y.I.; Berezina, N.A. The causes and consequences of algal blooms: The *Cladophora glomerata* bloom and the Neva estuary (eastern Baltic Sea). *Mar. Pollut. Bull.* **2010**, *61*, 183–188. [CrossRef]
- 9. Prazukin, A.; Shadrin, N.; Balycheva, D.; Firsov, Y.; Lee, R.; Anufriieva, E. *Cladophora* spp. (Chlorophyta) modulate environment and create a habitat for microalgae in hypersaline waters. *Eur. J. Phycol.* **2021**, *56*, 231–243. [CrossRef]
- 10. Gorbunova, Y.A.; Esyukova, E.E. Emissions of macroalgae and sea grasses in the Russian part of the South-Eastern coast of the Baltic Sea. *Izv. Kaliningr. State Tech. Univ.* **2020**, *59*, 24–34. (In Russian) [CrossRef]
- 11. Ebadi, A.G.; Hisoriev, H. The prevalence of heavy metals in *Cladophora glomerata* L. from Farahabad Region of Caspian Sea–Iran. *Toxicol. Environ. Chem.* **2017**, *99*, 883–891. [CrossRef]
- 12. Franzen, D.; Infantes, E.; Grondahl, F. Beach-cast as biofertiliser in the Baltic Sea region-potential limitations due to cadmiumcontent. *Ocean Coast. Manag.* 2019, 169, 20–26. [CrossRef]
- Gubelit, Y.; Polyak, Y.; Dembska, G.; Pazikowska-Sapota, G.; Zegarowski, L.; Kochura, D.; Krivorotov, D.; Podgornaya, E.; Burova, O.; Maazouzi, C. Nutrient and metal pollution of the eastern Gulf of Finland coastline: Sediments, macroalgae, microbiota. *Sci. Total Environ.* 2016, 550, 806–819. [CrossRef]
- 14. Bonsdorff, E. Drifting algae and zoobenthos—Effects on settling and community structure. *Neth. J. Sea Res.* **1992**, 30, 57–62. [CrossRef]
- 15. Hou, J.Z.; Wei, Q.; Gao, L.; Sun, W.M. Influence of decomposition of *Cladophora* sp. on phosphorus concentrations and forms in the overlying water. *Huan Jing Ke Xue* **2013**, *34*, 2184–2190. (In Chinese)
- 16. Kononenko, G.P.; Burkin, A.A.; Georgiev, A.A.; Georgieva, M.L. Mycotoxins in macroalgae from the Velikaya Salma Strait of the Kandalaksha Bay of the White Sea. *Biol. Morya* 2022, *48*, 53–61. (In Russian) [CrossRef]
- 17. Lenzi, M.; Cianchi, F. Summer dystrophic criticalities of non-tidal lagoons: The case study of a Mediterranean lagoon. *Diversity* **2022**, *14*, 771. [CrossRef]
- 18. Stevenson, R.J.; Bennett, B.J.; Jordan, D.N.; French, R.D. Phosphorus regulate stream injury by filamentous green algae, DO, and pH with thresholds in responses. *Hydrobiologia* **2012**, *695*, 25–42. [CrossRef]
- 19. Valiela, I.; McClelland, J.; Hauxwell, J.; Behr, P.; Hersh, D.; Foreman, K. Macroalgal blooms in shallow estuaries: Controls and ecophysiological and ecosystem consequences. *Limnol. Oceanogr.* **1997**, *42*, 1105–1118. [CrossRef]

- 20. Jones, M.; Pinn, E. The impact of a macroalgal mat on benthic biodiversity in Poole Harbour. *Mar. Poll. Bull.* **2006**, 53, 63–71. [CrossRef] [PubMed]
- Rivers, J.S.; Peckol, P. Interactive Effects of nitrogen and dissolved inorganic carbon on photosynthesis, growth, and ammonium uptake of the macroalgae *Cladophora vagabunda* and *Gracilaria tikvahiae*. Mar. Biol. 1995, 121, 747–753. [CrossRef]
- 22. Wan, A.H.; Wilkes, R.; Heesch, S.; Bermejo, R.; Johnson, M.; Morrison, L. Assessment and characterisation of Ireland's green tides (*Ulva species*). *PLoS ONE* **2017**, *12*, e0169049. [CrossRef] [PubMed]
- 23. Umanzor, S.; Ladah, L.; Calderon-Aguilera, L.E.; Zertuche-González, J.A. Intertidal macroalgae influence macroinvertebrate distribution across stress scenarios. *Mar. Ecol. Prog. Ser.* 2017, *584*, 67–77. [CrossRef]
- 24. Dolbeth, M.; Pardal, M.A.; Lillebo, A.I.; Azeiteiro, U.; Marques, J.C. Short- and long-term effects of eutrophication on the secondary production of an intertidal macrobenthic community. *Mar. Biol.* **2003**, *143*, 1229–1238. [CrossRef]
- 25. Berezina, N.A. Spatial distribution of macrofauna in a littoral zone with drifting macroalgae in the Neva estuary. *Est. J. Ecol.* **2008**, 57, 198–213. [CrossRef]
- 26. Page, M.; Goldhammer, T.; Hilt, S.; Tolentino, S.; Brothers, S. Filamentous algae blooms in a large, clear-water lake: Potential Drivers and Reduced Benthic Primary Production. *Water* **2022**, *14*, 2136. [CrossRef]
- Golubkov, S.M.; Berezina, N.A.; Gubelit, Y.I.; Demchuk, A.S.; Golubkov, M.S.; Tiunov, A.V. A relative contribution of carbon from green tide algae *Cladophora glomerata* and *Ulva intestinalis* in the coastal food webs in the Neva Estuary (Baltic Sea). *Mar. Pollut. Bull.* 2018, 126, 43–50. [CrossRef]
- Lauringson, V.; Kotta, J. Influence of the thin drift algal mats on the distribution of macrozoobenthos in Koiguste Bay, NE Baltic Sea. *Hydrobiologia* 2006, 554, 97–105. [CrossRef]
- Tomczak, M.T.; Müller-Karulis, B.; Järv, L.; Kotta, J.; Martin, G.; Minde, A.; Põllumäe, A.; Razinkovas, A.; Strake, S.; Bucas, M.; et al. Analysis of trophic networks and carbon flows in south-eastern Baltic coastal ecosystems. *Prog. Oceanogr.* 2009, 1–4, 111–131. [CrossRef]
- Razinkovas-Baziukas, A.; Morkūnė, R.; Bacevičius, E.; Gasiūnaitė, Z.R. Trophic network model of exposed sandy coast: Linking continental and marine water ecosystems. *Estuar. Coast. Shelf Sci.* 2017, 195, 110–123. [CrossRef]
- Golterman, H.L. (Ed.) Methods for Chemical Analysis of Freshwaters. IBP Handbook 8; Blackwell Scientific Publ.: Oxford, UK; Edinburgh, UK, 1969; 172p.
- 32. Berezina, N.; Strode, E.; Lehtonen, K.; Balode, M.; Golubkov, S. Sediment quality assessment using *Gmelinoides fasciatus* and *Monoporeia affinis* (Amphipoda, Gammaridea) in the northeastern Baltic Sea. *Crustaceana* **2013**, *86*, 780–801. [CrossRef]
- Berezina, N.A.; Gubelit, Y.I.; Polyak, Y.M.; Sharov, A.N.; Kudryavtseva, V.A.; Lubimtsev, V.A.; Petukhov, V.A.; Shigaeva, T.D. An integrated approach to the assessment of the eastern Gulf of Finland health: A case study of coastal habitats. *J. Mar. Syst.* 2017, 171, 159–171. [CrossRef]
- 34. Phillips, D.L.; Cregg, J.W. Source Partitioning Using Stable Isotopes: Coping with Too Many Sources. *Oecologia* 2003, 136, 261–269. [CrossRef]
- 35. Moore, J.W.; Semmens, B.X. Incorporating uncertainty and prior information into stable isotope mixing models. *Ecol. Lett.* **2008**, 11, 470–480. [CrossRef]
- 36. Parnell, A.C.; Phillips, D.L.; Bearhop, S.; Semmens, B.X.; Ward, E.J.; Moore, J.W.; Jackson, A.L.; Grey, J.; Kelly, D.J.; Inger, R. Bayesian stable isotope mixing models. *Environmetrics* **2013**, *24*, 387–399. [CrossRef]
- 37. Stock, B.C.; Jackson, A.L.; Ward, E.J.; Parnell, A.C.; Phillips, D.L.; Semmens, B.X. Analyzing mixing systems using a new generation of Bayesian tracer mixing models. *PeerJ* 2018, *6*, e5096. [CrossRef] [PubMed]
- Fong, J.M.; Lai, S.; Yaakub, S.M.; Ow, Y.X.; Todd, P.A. The diet and feeding rates of gastropod grazers in Singapore's seagrass meadows. *Bot. Mar.* 2018, *61*, 181–192. [CrossRef]
- Mordukhovich, V.; Fadeeva, N.; Kiyashko, S. Use of analysis of stable isotope ratios and fatty acid composition in trophoecological studies of marine free-living nematodes. In *Studies of Marine Organisms in the Far East: Biodiversty, Monitoring, and Rational Management of Resources*; Malakhov, V.V., Chernyshev, A.V., Eds.; Far Eastern Federal University Press: Vladivostok, Russia, 2020; pp. 307–346.
- 40. Berezina, N.A.; Golubkov, S.M.; Gubelit, J.I. Grazing effects of alien amphipods on macroalgae in the littoral zone of the Neva Estuary (Eastern Gulf of Finland, Baltic Sea). *Oceanol. Hydrobiol. Stud.* **2005**, *34* (Suppl. S1), 63–82.
- 41. Best, R.; Chaudoin, A.L.; Bracken, M.E.S.; Graham, M.H.; Stachowicz, J.J. Plant-animal diversity relationships in a rocky intertidal system depend on invertebrate body size and algal cover. *Ecology* **2014**, *95*, 1308–1322. [CrossRef] [PubMed]
- 42. Dean, R.L.; Connell, J.H. Marine invertebrates in an algal succession. I. Variations in abundance and diversity with succession. *J. Exp. Mar. Biol. Ecol.* **1987**, *109*, 195–215. [CrossRef]
- Duffy, J.E.; Hay, M.E. Strong impacts of grazing amphipods on the organization of a benthic community. *Ecol. Monogr.* 2000, 70, 237–263. [CrossRef]
- 44. Lotze, H.K.; Schramm, W.; Schories, D.; Worm, B. Control of macroalgal blooms at early developmental stages: *Pilayella littoralis* versus *Enteromorpha* spp. *Oecologia* **1999**, *119*, 46–54. [CrossRef]
- 45. Menge, B.A.; Daley, B.A.; Wheeler, P.A.; Strub, P.T. Rocky intertidal oceanography: An association between community structure and nearshore phytoplankton concentration. *Limnol. Oceanogr.* **1997**, *42*, 57–66. [CrossRef]

- Kharlamenko, V.I.; Kiyashko, S.L.; Imbs, A.B.; Vyshkvartzev, D.I. Identification of food sources of invertebrates from the seagrass *Zostera marina* community using carbon and sulfur stable isotope ratio and fatty acid analyses. *Mar. Ecol. Progr. Ser.* 2001, 220, 103–117. [CrossRef]
- Ponsard, S.; Arditi, R. What can stable isotopes (δ¹⁵N and δ¹³C) tell about the food web of soil macro-invertebrates? *Ecology* 2000, *81*, 852–864. [CrossRef]
- 48. Peterson, B.J.; Fry, B. Stable Isotopes in Ecosystem Studies. Annu. Rev. Ecol. Syst. 1987, 18, 293–320. [CrossRef]
- 49. Hemminga, M.A.; Mateo, M.A. Stable carbon isotopes in seagrasses: Variability in ratios and use in ecological studies. *Mar. Ecol. Prog. Ser.* **1996**, *140*, 285–298. [CrossRef]
- 50. Gladyshev, M.I. Stable isotope analyses in aquatic ecology (a review). J. Sib. Fed. University. Biol. 2009, 2, 381–402.
- Fry, B.; Arnold, C. Rapid ¹³C/¹²C turnover during growth of brown shrimp (*Penaeus aztecus*). Oecologia 1982, 5, 200–204. [CrossRef]
- 52. Stephenson, R.L.; Tan, F.C.; Mann, K.H. Stable carbon isotope variability in marine macrophytes and its implications for food web studies. *Mar. Biol.* **1984**, *81*, 223–230. [CrossRef]
- McMillan, C.; Smith, B.N. Comparison of δ¹³C values for seagrasses in experimental cultures and in natural habitats. *Aquat. Bot.* 1982, 14, 381–387. [CrossRef]
- 54. Fry, B.; Sherr, E.B. δ¹³C measurements as indicators of carbon flow in marine and freshwater ecosystems. *Contrib. Mar. Sci.* **1984**, 27, 13–47.
- 55. Mercado, J.M.; de los Santos, C.B.; Lucas Pérez-Lloréns, J.; Vergara, J.J. Carbon isotopic fractionation in macroalgae from Cádiz Bay (Southern Spain): Comparison with other bio-geographic regions. *Estuar. Coast. Shelf Sci.* **2009**, *85*, 449–458. [CrossRef]
- 56. Michener, R.H.; Schell, D.M. Stable isotope ratios as tracers in marine aquatic food webs. In *Stable Isotopes in Ecology and Environmental Science*; Lajtha, K., Michener, R.H., Eds.; Blackwell Science: Oxford, UK, 1994; pp. 138–157.
- 57. Hardison, A.K.; Canuel, E.A.; Anderson, I.C.; Veuger, B. Fate of macroalgae in benthic systems: Carbon and nitrogen cycling within the microbial community. *Mar. Ecol. Prog. Ser.* **2010**, *414*, 41–55. [CrossRef]
- Shafique, S.; Siddiqui, P.; Aziz, R.; Shoaib, N. Variations in carbon and nitrogen contents during decomposition of three macroalgae inhabiting sandspit backwater, Karachi. *Pak. J. Bot.* 2013, 45, 1115–1118.
- Veuger, B.; Eyre, B.D.; Maher, D.; Middelburg, J.J. Nitrogen incorporation and retention by bacteria, algae, and fauna in a subtropical intertidal sediment: An in situ ¹⁵N labeling study. *Limnol. Oceanogr.* 2007, 52, 1930–1942. [CrossRef]
- 60. Choo, K.S.; Pedersén, M.; Snoeijs, P. Uptake of inorganic carbon by *Cladophora glomerata* (Chlorophyta) from the Baltic Sea. *J. Phycol.* **2002**, *38*, 493–502. [CrossRef]
- 61. Paalme, T.; Kukk, H.; Kotta, J.; Orav, H. "In vitro" and "in situ" decomposition of nuisance macroalgal *Cladophora glomerata* and *Pilayella littoralis*. *Hydrobiologia* **2002**, 475, 469–476. [CrossRef]
- 62. Sand-Jensen, K.; Borum, J. Interactions among phytoplankton, periphyton, and macrophytes in temperate freshwaters and estuaries. *Aquatic Botany* **1991**, *41*, 137–175. [CrossRef]
- 63. Gubelit, Y.I.; Makhutova, O.N.; Sushchik, N.N.; Kolmakova, A.A.; Kalachova, G.S.; Gladyshev, M.I. Fatty acid and elemental composition of littoral "green tide" algae from the Gulf of Finland, the Baltic Sea. J. Appl. Phycol. 2015, 27, 375–386. [CrossRef]