diversity

MDPI

Article

Bioenergetics of *Euphausia superba* and *Euphausia crystallorophias* in the Ross Sea

Andrea De Felice *, Elena Manini, Ilaria Biagiotti and Iole Leonori

National Research Council (CNR), IRBIM—Institute for Marine Biological Resources and Biotechnologies, Largo Fiera della Pesca, 1, 60125 Ancona, Italy
* Correspondence: andrea.defelice@cnr.it

Abstract: Krill species are key organisms in the Antarctic food web. Biochemical composition in terms of lipids, proteins, carbohydrates, and fatty acids and its implications for spatial distribution were investigated in specimens of *Euphausia superba* and *Euphausia crystallorophias* collected in the Ross Sea and the adjacent Pacific region during an acoustic survey carried out within the framework of the 19th Italian National Program for Research in Antarctica (PNRA) Expedition, to gain insights into their trophic relationships and bioenergetic strategies. In both species, the body biochemical composition (wet) showed a predominance of proteins (62–86%), followed by lipids and carbohydrates, and, among identified lipid classes, the two species did not seem to differ much in fatty acid composition. Results showed the highest dissimilarity in biochemical composition between species relative to differences in latitude (24%) and to inshore/offshore haul (22%). Fatty acid analysis, and particularly PUFA/SFA and 18PUFA/16PUFA ratios, allowed identification of a more pronounced omnivorous kind of diet in *E. crystallorophias* relative to *E. superba*.

Keywords: bioenergetics; *Euphausia superba*; *Euphausia crystallorophias*; biochemical composition; fatty acids; Ross Sea

check for updates

Citation: De Felice, A.; Manini, E.; Biagiotti, I.; Leonori, I. Bioenergetics of *Euphausia superba* and *Euphausia crystallorophias* in the Ross Sea. *Diversity* **2023**, *15*, 480. https://doi.org/10.3390/d15040480

Academic Editor: Michael Wink

Received: 15 February 2023
Revised: 20 March 2023
Accepted: 21 March 2023
Published: 24 March 2023

1. Introduction

The Ross Sea supports a large concentration of Antarctic krill (*Euphausia superba*) and ice krill (*Euphausia crystallorophias*), which play a key ecological role constituting a fundamental link between primary producers (phytoplankton) and top predators [1–3]. It has been estimated that the total consumption of krill by marine mammals is around 10–20 million tons/year in the North Pacific, 15–25 million tons/year in the North Atlantic, and 125–250 million tons/year in the Southern Hemisphere, with the biggest part played in the Southern Ocean [4]; these numbers give an idea of the importance of this pelagic resource in Antarctic waters. Moreover, krill is involved in biogeochemical cycles, and, given their large size, high biomass, and daily vertical migrations, they are an important vehicle of transport and transformation of essential nutrients, stimulating primary productivity and influencing carbon sink [5]. In time, such valuable marine resources have developed strategies of adaptation to the extreme conditions in Antarctica involved in particular their diet [6]. The Euphausiids living in Antarctic waters share a predominantly herbivorous diet, with some differences among species. Bacteria may play an important role in the diet of *E. superba* [7]; high bacterial concentrations described in the stomach suggest that they may be part of the microbiota [8], providing amino acids, enzymes, and vitamins. Adverse environmental conditions may induce dietary changes [9,10]; for instance, in winter adults may eat detritus and heterotrophic material [11,12].

Starving krill slow their metabolic rate and feed on body protein [13], as inferred from the C:N ratio, resulting in body shrinkage. Lipids in the form of triacylglycerols are used as an energy source through the winter [14,15]; however, they provide only 11% of the energy for successful overwintering, while reduction of the metabolic rate accounts

for 71% [15]. A study comparing krill lipid content in autumn and winter has reported somewhat conflicting evidence that can probably be explained by differences between open water and pack ice sampling sites and by high inter-individual variability [16].

Lipid storage strategies differ significantly among krill species. Extensive lipid reserves (triacylglycerol and phosphatidylcholine) allow *E. superba* to survive the Antarctic winter, whereas *E. crystallorophias* and *Thysanoessa macrura* rely on wax esters and phosphatidylcholine [17,18]. It has also been reported that lipid accumulation by *E. superba* during the productive period and lipid consumption in winter/spring before the phytoplankton bloom are much more pronounced than previously assumed [14]. The substantial lipid accumulation by this biomass species clearly has strong implications for the biogenic energy fluxes in Antarctic waters. Notably, lipids are not only energy reserves, but they also reduce energy dispersion during vertical transfers in the water column, favouring buoyancy [19–21].

Data on lipid amount and composition in krill species in different areas and environmental conditions are not very common. Hellessey et al. [22] described some regional differences among Antarctic krill populations in Atlantic, Indian, and Pacific sectors of the Southern Ocean, finding that Indian sector krill presented a different dietary lipid pattern relative to Atlantic and Pacific sector krill by examining their fatty acid profiles; moreover, the krill diet from the Indian sector was characterized more by copepods and diatomeae. Ju and Harvey [23] have described an interesting difference in total lipids between adult individuals of *E. superba* (20.2%) and *E. crystallorophias* (30%) caught in the vicinity of Adelaide Island. In *E. superba*, 45.5% of lipids were triacylglycerols, whereas in *E. crystallorophias* more than half were wax esters (55.9%); in both species, a higher content of polyunsaturated fatty acids (PUFAs) was found in larvae compared with adults. Where differences in fatty acid (FA) and sterol content were not significant, greater amounts of fatty alcohols (14:0, 16:0), which are needed for wax ester biosynthesis, were found in *E. crystallorophias*. FA and gut content analysis highlighted a greater reliance on lipid reserves by the latter species for overwintering [23].

This paper analyses the biochemical composition in terms of lipid, protein, and carbohydrate contents and fatty acid composition of *E. superba* and *E. crystallorophias* from the Ross Sea to reveal differences that could be associated with different dietary strategies and thus different ways of adapting to the surrounding environment. These differences could also be associated with the main food source for each species and consequently with their different geographic localization in the Ross Sea [2,24].

2. Materials and Methods

2.1. Study Area and Sampling

E. superba and *E. crystallorophias* specimens were collected in the western sector of the Ross Sea (Antarctica) (hauls 7, 8, 10, 13, and 17) and the adjacent Pacific area (hauls 1, 26, 27, 28, and 30) in January 2004, during an oceanographic cruise aboard *R/V Italica*. The study was conducted within the framework of the 19th Italian National Program for Research in Antarctica (PNRA) Expedition. The area boundary coordinates are 69° and 76° S latitude and 165° E and 175° W longitude. The top 200 m of the pelagic domain were studied using an acoustic survey. Krill swarms were monitored acoustically at three frequencies (38, 120, and 200 kHz), to maximize species discrimination, and using periodical trawling to obtain ground truth data. Bottom depth ranged from about 300 to 3500 m, the northern area at the border between Ross Sea and the Southern Ocean being the deepest.

Specimens were collected with target fishing mainly using the HPRI-1000 plankton net (mesh size 1 mm), which was designed by CNR-IRBIM (Ancona, Italy), and was largely based on the observation of krill swarms by acoustic surveys [25]. The geographical position of each haul is reported in Figure 1. Haul duration was about 30 min; after net recovery, all krill specimens, or a subsample in case of huge catches, were sorted, measured, and weighed as quickly as possible. Some specimens were then frozen at −20 °C.

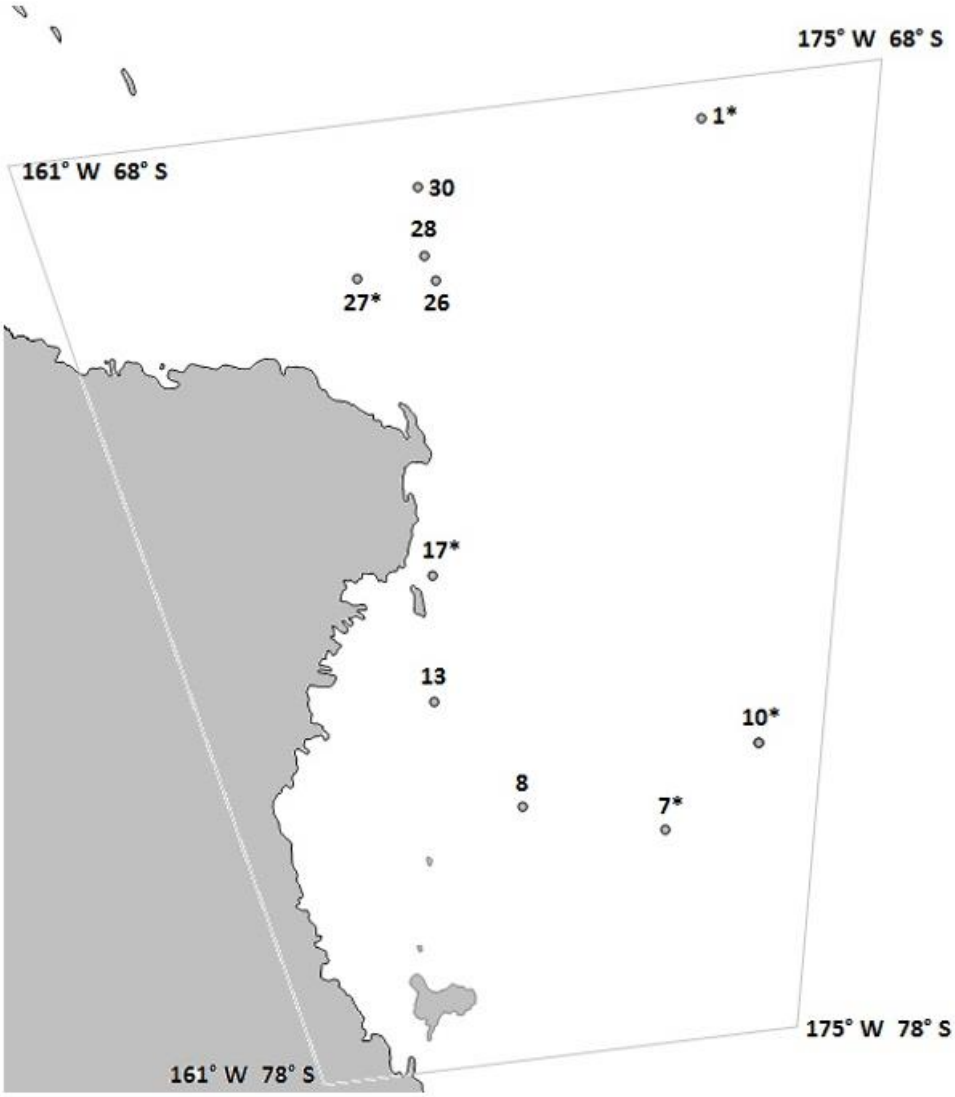


Figure 1. Krill sampling stations. Asterisks indicate the hauls that provided the specimens subjected to fatty acid analysis.

Main information concerning haul locations is reported in Table 1.

Table 1. Geographical location of the hauls in western Ross Sea and the adjacent Pacific Ocean: latitude, longitude, water depth, maximum fishing net depth, and main environmental variables.

Haul	Lat.	Long.	Water Depth (m)	Net Depth (m)	Ice Cover (%)
1	68°42.3′ S	179°53.1′ W	3438.5	120	30
7	75°56.4′ S	178°30.9′ E	540.0	100	20
8	75°36.0′ S	172°39.8′ E	555.5	100	0
10	75°6.59′ S	177°24.5′ W	450.9	37	10
13	74°27.1′ S	170°0.35′ E	578.3	125	0
17	73°8.8′ S	170°44.4′ E	512.6	75	0
26	70°10.6′ S	171°56.8′ E	1479.0	40	0
27	70°2.69′ S	169°54.0′ E	2662.5	21	0
28	69°54.1′ S	172°0.95′ E	1863.0	35	0
30	69°11.7′ S	171°47.8′ E	3043.5	64	0

2.2. Biometric Measurements

Morphometric characteristics were assessed in specimens preserved at −20 °C. Total length (TL, from the tip of the rostrum to the tip of the telson) and carapax length (CL, from the tip of the rostrum to the posterior margin of the carapax) were measured to the nearest 0.05 mm using a calliper. The wet weight of soft tissue was measured to the nearest 0.0001 g after removing the exoskeleton; the soft tissue was then used for biochemical analyses.

2.3. Biochemical Analyses

For the biochemical analyses, 20 individuals of *E. superba* and 20 of *E. crystallorophias* per haul were used to determine percent lipids, carbohydrates, and proteins in known amounts of soft tissue (wet weight, WW). Soft tissue from each individual, after the removal of the exoskeleton, was homogenized in 0.9% NaCl solution (final volume, 2 mL) and subsequently ensonified. Each analysis used 100 µL of homogenised tissue.

Total protein was determined using Hartree's colorimetric method [26] adapted to crustaceans [27], using the modified Lowry protein assay [28].

Total carbohydrates were determined according to Dubois et al. [29], using a method applied to sediment by Gerchakov and Hatcher [30].

Total lipids were determined according to the method of Bligh–Dyer [31], adapted to crustaceans [27].

The FA composition of lipid extracts was analysed using gas chromatography; total lipid content was determined according to Folch et al. [32], with some modifications. The krill sample (0.5–1 g), placed in a Falcon test tube, was mixed with 16 mL of a 1:1 (*v*/*v*) chloroform:methanol solution and homogenized with Ultraturrax (IKA, Staufen, Germany) for 3 min. Then, 8 mL of chloroform were added, and after having been homogenized for 2 min, the mixture was filtered through a Buchner filter, through the vacuum created by the water pump. The filtrate, once collected in a flask, was transferred into a Falcon test tube and was mixed with 6 mL of aqueous solution of KCl 1M. After being stirred for a minute with vortexing, the aqueous phase and the organic phase were separated using centrifugation (3000 revolutions/minute, time = 15 min). Then, the organic phase (below) was recovered, which was then filtered through a pleated filter, in a 50 mL flask. Finally, the solvent was removed from the organic phase using a rotary evaporator under vacuum in a thermostatic bath at 40 °C. Methyl esters of fatty acids (FAMEs) of total lipids were obtained according to Christie [33]. These analyses used specimens from 5 hauls (marked with an asterisk in Figure 1) [34].

Hauls were compared in pairs to determine which conditions yielded the largest differences between species (*E. superba* vs. *E. crystallorophias*), geographical position of the same species (Ross Sea vs. Southern Ocean), and distance from the coast of the same species (inshore vs. offshore). A larger number of *E. crystallorophias* individuals per haul had to be analysed due to their smaller mean size and to the fact that at least 0.5 g of fresh tissue are needed to provide reliable data. As regards *E. superba*, FA classes were analysed separately in adults and subadults (<35 mm) from hauls n. 1 and 27, to seek differences between them.

2.4. Statistical Analysis

A number of statistical tests were applied to the data by means of SPSS software. First of all, one-way ANOVA and Newman–Keuls test were applied to *E. superba* and *E. crystallorophias* specimens from haul n. 10, the only one that had provided a sufficient number of individuals of both species, to analyse differences in size and biochemical composition, due to the satisfied criterion of normality of the distributions.

The Kruskal–Wallis test was applied to measure intraspecies differences in biochemical composition in specimens of different length classes from different hauls, to learn whether composition varies between sites and during growth; this test was selected because its proper use foresees series of measurements of individuals of the same population.

Mann–Whitney's test was instead used to compare biochemical composition between species, as this test is mainly used to compare individuals of independent groups (species in this case).

SIMPER analysis was applied to establish in detail whether differences in biochemical composition were due to species-specific or geographical-environmental factors. Its results were tested using ANOSIM. MDS analysis enabled a clearer identification of the groups resulting from biochemical composition analyses.

Finally, the Mann–Whitney test was applied to FA data to assess the presence and possible significance of species-specific differences between *E. superba* and *E. crystallorophias*; this test was chosen because it is more suitable in a specific case such as this where a group of individuals were used together to reach a minimum critical weight needed to perform the analysis, resulting in only one sample per haul for the seven hauls selected for analysis.

3. Results

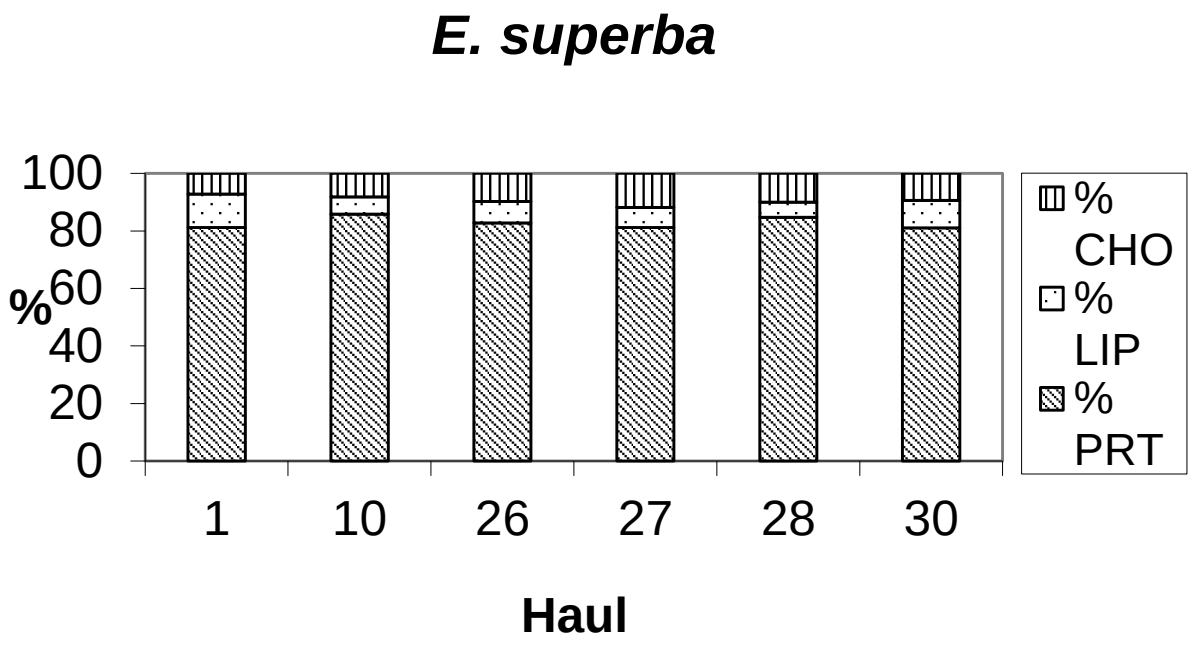
3.1. Biochemical Composition of E. superba and E. crystallorophias

A total of 220 krill individuals obtained from 10 hauls conducted in the western Ross Sea and the adjacent Pacific Ocean area were analysed. The krill assemblages sampled can be considered as representative of the whole krill population in the area [35,36]. A different distribution of the two species was documented, as reported in earlier studies [2,24,35,37,38]. Individuals from different hauls exhibited considerable total mean length homogeneity (*E. superba*, 42 mm; *E. crystallorophias*, 22 mm; Table 2), probably due to the scarcity of juvenile specimens in the samples. Although juveniles are identified by sexual development stage, they generally measure up to 25–30 mm in *E. superba* and 15–20 mm in *E. crystallorophias* [35,39,40].

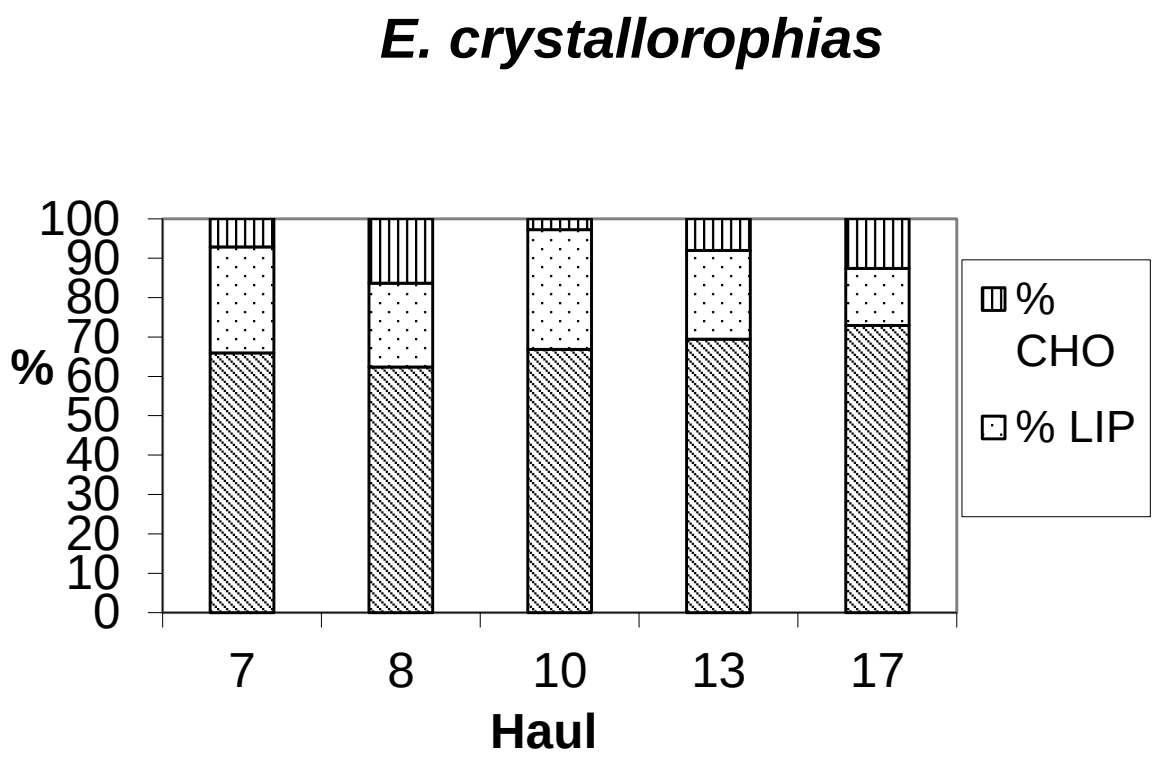
Table 2. Mean total length ± standard deviation of *E. superba* and *E. crystallorophias* specimens and percent content in Total Organic Matter (TOM) and ash relative to the wet weight of each organism. No. = number of analysed samples, σ_{TL} = standard deviation, TL = total length, WW = wet weight, TOM = Total Organic Matter, na = not available.

Species	Haul	No.	Mean TL (mm)	σ_{TL} (mm)	Mean WW (g)	σWW (g)	TOM (%)	Ash (%)
E. superba	1	20	42.94	3.72	0.1113	0.0283	na	na
E. superba	10	20	39.92	4.90	0.1156	0.0441	na	na
E. superba	26	20	41.75	4.01	0.1277	0.0481	84.9	15.1
E. superba	27	20	41.82	2.46	0.1026	0.0268	81.8	18.2
E. superba	28	20	40.56	2.29	0.1115	0.0348	88.4	11.6
E. superba	30	20	42.48	2.50	0.1233	0.0383	82.9	17.1
E. cryst.	7	20	24.12	1.82	0.0281	0.0171	86.4	13.6
E. cryst.	8	20	22.35	2.22	0.0167	0.0093	86.0	14.0
E. cryst.	10	20	24.85	3.04	0.0224	0.0087	na	na
E. cryst.	13	20	22.84	3.30	0.0258	0.0157	88.1	11.9
E. cryst.	17	20	23.26	3.28	0.0138	0.0055	82.0	18.0

In both species (Figure 2), proteins were the predominant biochemical class of organic compounds (62–86%), followed by lipids in *E. crystallorophias* (23 ± 3%) and by carbohydrates in *E. superba* (9.4 ± 0.7%). The protein/carbohydrate ratio was >1 in all specimens. The biochemical composition of organic matter (OM; Figure 3) showed large quantitative differences with higher protein and lipid concentrations in *E. crystallorophias*

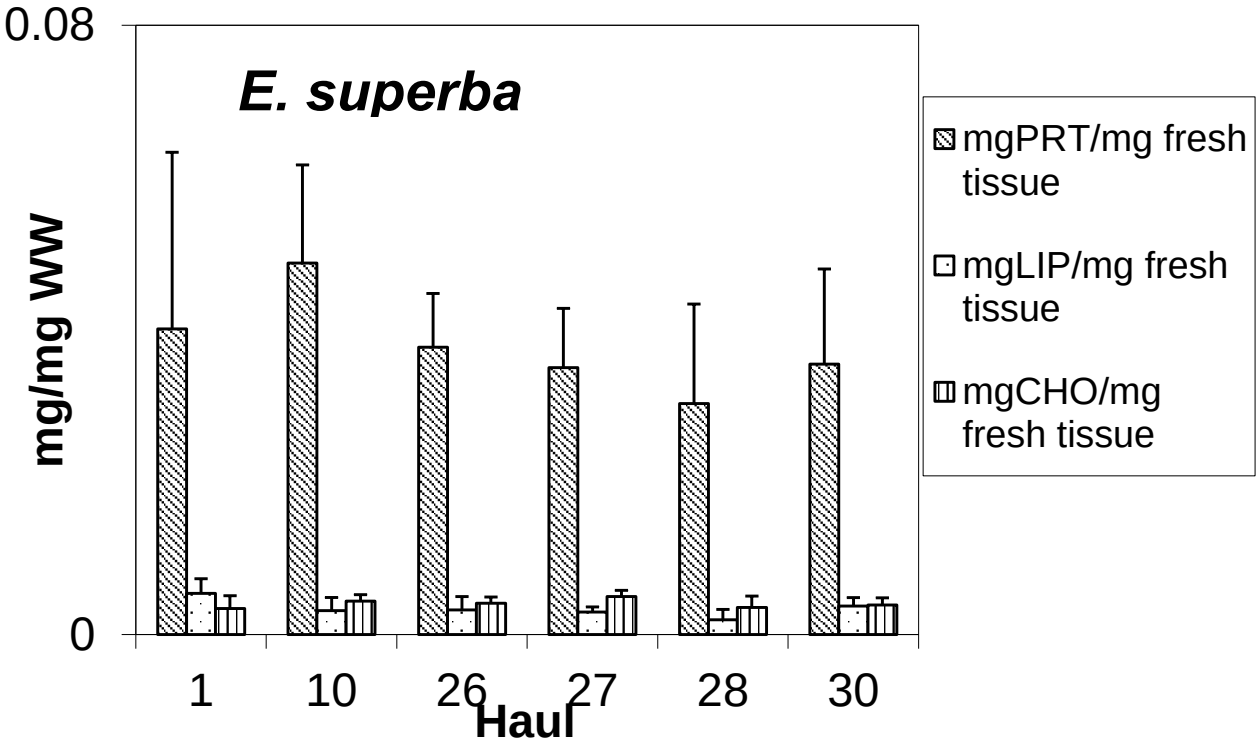


(**a**)

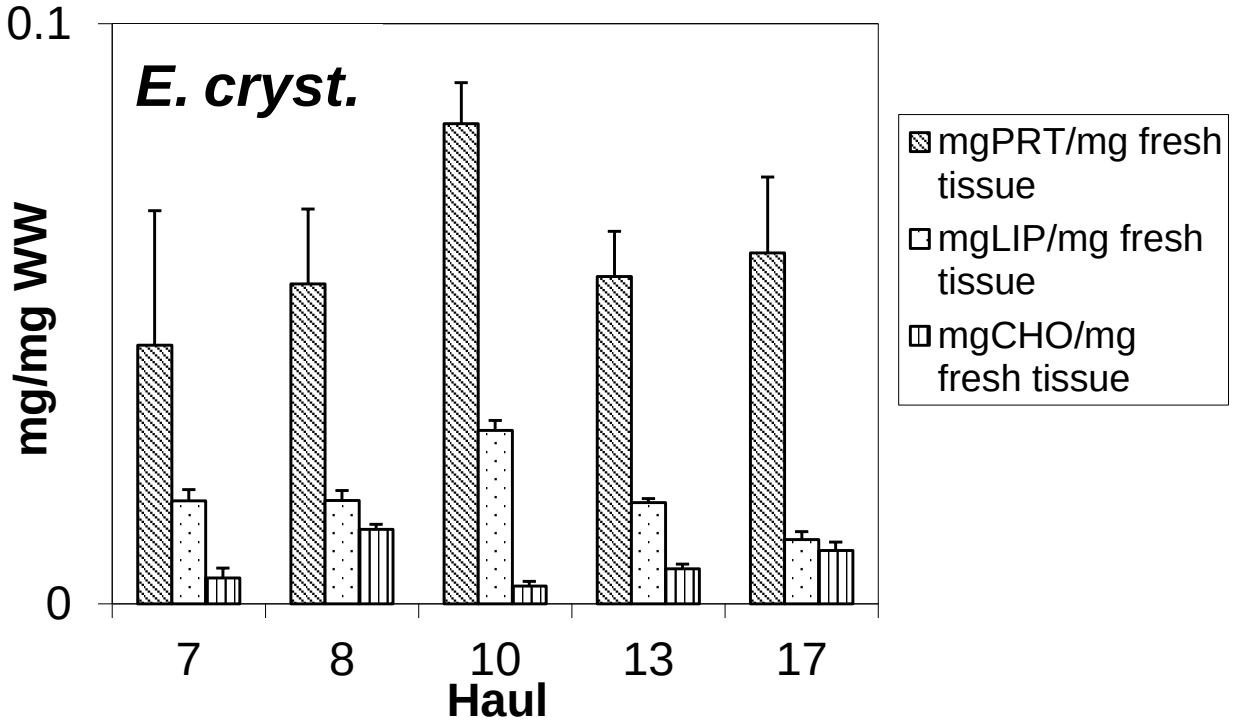


(**b**)

Figure 2. (**a**) Percent biochemical composition of *E. superba* in the selected hauls; (**b**) percent biochemical composition of *E. crystallorophias* in the selected hauls. CHO = carbohydrates, LIP = lipids, PRT = proteins.



(**a**)



(**b**)

Figure 3. (**a**) Mean content in lipids, proteins, and carbohydrates (mg/mg wet weight) with standard deviation intervals in the selected hauls for *E. superba*; (**b**) Mean content in lipids, proteins, and carbohydrates (mg/mg wet weight) with standard deviation intervals in the selected hauls for *E. crystallorophias*. CHO = carbohydrates, LIP = lipids, PRT = proteins, WW = wet weight.

The biochemical composition (in mg/mg of fresh tissue) and size of specimens of both krill species collected at haul n. 10 were analysed using one-way ANOVA and Newman–

Keuls test, which highlighted significant differences in lipids, carbohydrate content, and TL, whereas differences in protein content were not significant (Table S1), showing evidence that the main differences between the two species in terms of biochemical composition are due to lipid and carbohydrate contents and not due to protein contents.

Data analysis of species using nonparametric Kruskal–Wallis test found significant differences among sampling hauls for total lipid and carbohydrate fresh content (mg/mg tissue) in *E. superba* and for all three compounds in *E. crystallorophias* (Table S1), demonstrating a good degree of variability for the same species among different sampling sites. Analysis by species and by size class (2 mm intervals) found a significant relationship only in *E. crystallorophias*, where carbohydrates fell with increasing TL (Table S1).

Mann–Whitney's test, applied to analyse the differences in biochemical composition between species, highlighted significant differences for all three parameters (Table S2). SIMPER (Table S3), ANOSIM (Table S4), and MDS (Figure 4) analysis were performed with PRIMER software [41], to establish the role of each factor in such differences.

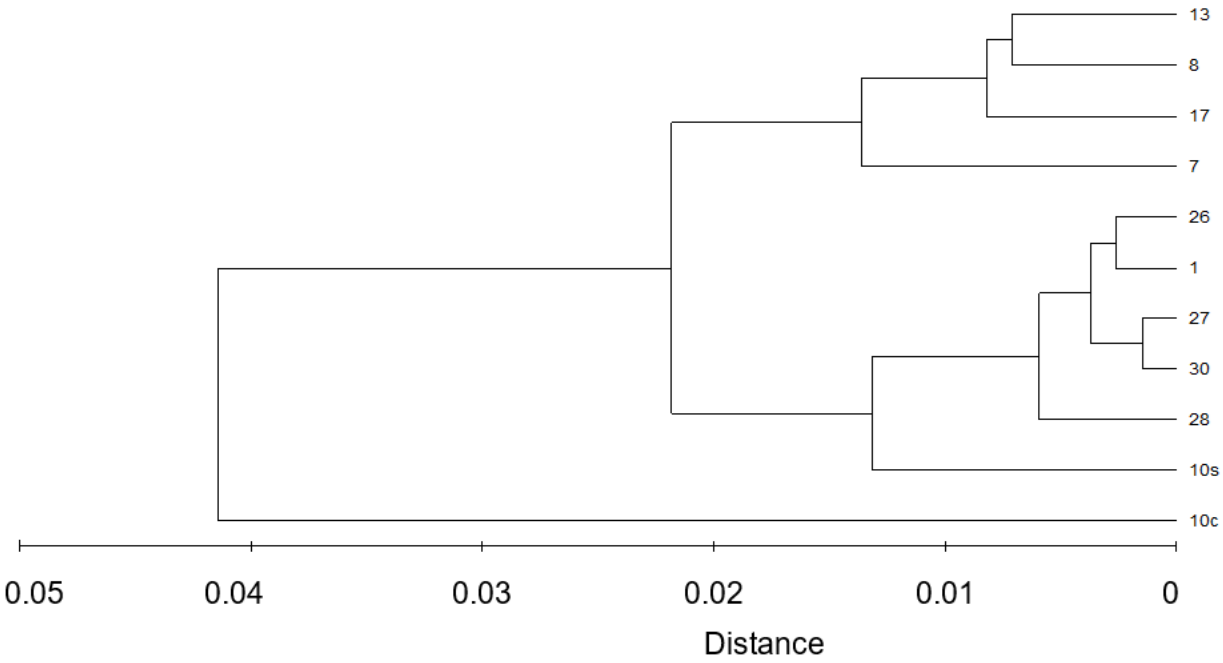


Figure 4. Differences concerning biochemical composition in the selected hauls. Dendrogram relative to MDS analysis applied on biochemical composition data from the two krill species. 10 s = *E. superba* individuals from haul n. 10; 10c = *E. crystallorophias* individuals from haul n. 10.

High similarity was found among individuals from the same haul (74–88%); dissimilarity was higher by species (average 34%) than by differences in latitude (24%) and distance from the coast (22%) (SIMPER, Table S3). MDS analysis found similar results and appeared to identify three groups in terms of biochemical composition (Figure 4): *E. crystallorophias* from haul n. 10 is the most diverse group; the other two groups are composed of *E. crystallorophias* from hauls n. 7, 8, 13, and 17 and by *E. superba* from hauls n. 1, 10, 26, 27, 28, and 30.

3.2. Fatty Acid Composition in E. superba and E. crystallorophias

The specimens used for FA analyses are described in Table 3, while FA classes and their percent amount are reported in Table 4 (*E. superba*) and Table 5 (*E. crystallorophias*).

Table 3. Krill specimens used for fatty acid characterisation, their length measurements, content in SFA, MUFA, and PUFA and PUFA/SFA ratio. TL = total length, σ_{TL} = standard deviation, SFA = saturated fatty acids, MUFA = mono-unsaturated fatty acids, PUFA = polyunsaturated fatty acids.

Species and Life Stage	Haul	Specimen No.	Mean TL (mm)	σ_{TL} (mm)	SFAs (%)	MUFAs (%)	PUFAs (%)	PUFAs/SFAs
E. superba adults	1	5	44.70	1.70	28.6	30.0	41.4	1.4
E. superba subadults	1	8	29.60	10.15	27.4	36.6	36.0	1.3
E. superba adults	10	13	40.85	4.82	36.3	30.3	33.4	0.9
E. superba adults	27	11	41.20	2.67	33.8	29.2	37.0	1.1
E. superba subadults	27	9	31.85	3.55	31.8	29.2	39.0	1.2
E. crystallorophias adults	7	25	24.85	2.58	16.4	49.3	34.3	2.1
E. crystallorophias adults	17	32	24.88	2.37	20.7	42.4	36.9	1.8

Table 4. Fatty acids classes and percent content found in *E. superba* individuals. At the bottom of the table the most relevant ratios, useful to characterize krill main diet, are reported. E. s. = *Euphausia superba*, PUFA = polyunsaturated fatty acids, SFA = saturated fatty acids, DHA = docosahexaenoic acid, EPA = eicosapentaenoic acid.

Fatty Acid	Haul 1 (E. s. Adults)	Haul 1 (E. s. Subadults)	Haul 10 (E. s. Adults)	Haul 27 (E. s. Adults)	Haul 27 (E. s. Subadults)
C14:0	6.0	5.6	10.4	8.7	9.2
C15:0	0.4	0.3	0.2	0.1	0.1
C16:0	21.0	19.7	23.7	23.1	20.8
C16:1ω7	3.4	3.3	5.3	3.9	4.1
C16:2	1.4	1.2	1.4	1.2	1.2
C18:0	1.3	1.8	2.0	1.9	1.7
C18:1ω9C	16.7	25.8	16.2	16.8	16.2
C18:1ω7C	8.5	6.1	7.1	7.2	7.4
C18:2ω6	3.4	3.8	2.4	2.1	1.8
C18:3ω6	0.1	0.2	0.4	0.6	0.7
C18:3ω3	0.7	0.5	0.8	0.8	0.8
C18:4ω3	2.6	2.4	5.5	5.3	6.2
C20:1ω9	1.1	1.1	1.3	0.9	1.2
C20:3ω6	0.5	0.5	0.6	0.4	0.3
C20:4ω6	0.8	0.7	0.4	0.6	0.6
C20:5ω3	16.7	13.6	11.8	12.5	12.8
C22:1ω9	0.2	0.2	0.4	0.4	0.4
C22:6ω3	15.2	13.1	10.0	13.5	14.5
PUFA/SFA	1.4	1.3	0.9	1.1	1.2
DHA/EPA	0.9	1.0	0.9	1.1	1.1
C18:4ω3/C16:1ω7	0.8	0.7	1.0	1.4	1.5
C18:1ω9C/C18:1ω7C	2.0	4.2	2.3	2.3	2.2
18PUFA/16PUFA	4.9	5.8	6.5	7.3	7.9

The major FA components found in *E. superba* and *E. crystallorophias* were the same as reported by Ju and Harvey [23], except for 18:4ω3 and 22:6ω3 (docosahexaenoic acid = DHA), which were abundant in our specimens and absent in those examined by the cited study. The same applies to the high concentrations of 14:0 measured in adult and sub-adult *E. superba* and of 18:1ω9C found in adult *E. crystallorophias*, as well as to the small amounts of 20:1 and 22:1 found in both species, even if the slightly higher values in *E. superba* for these two last isomers could indicate bigger quantities of copepods in its diet. Schaafsma et al. [16] found that the most represented fatty acids in age class 0 *E. superba* were 16:0, 22:6ω3, and 20:5ω3 (eicosapentaenoic acid = EPA); similar to the findings of this work, these two last compounds are the basic components of phospholipids.

Table 5. Fatty acids classes and percent content found in *E. crystallorophias* individuals. At the bottom of the table the most relevant ratios, useful to characterize krill main diet, are reported. E. c. = *Euphausia crystallorophias*, PUFA = polyunsaturated fatty acids, SFA = saturated fatty acids, DHA = docosahexaenoic acid, EPA = eicosapentaenoic acid.

Fatty Acid	Haul 7 (E. c. Adults)	Haul 17 (E. c. Adults)
C14:0	2.0	3.7
C15:0	0.1	0.1
C16:0	13.2	15.9
C16:1ω7	4.1	4.5
C16:2	0.4	0.4
C18:0	1.0	1.0
C18:1ω9C	32.5	26.9
C18:1ω7C	12.0	10.1
C18:2ω6	2.0	1.9
C18:3ω6	0.4	0.4
C18:3ω3	0.3	0.4
C18:4ω3	2.4	2.0
C20:1ω9	0.6	0.8
C20:3ω6	0.2	0.3
C20:4ω6	0.6	0.7
C20:5ω3	15.7	16.4
C22:1ω9	0.1	0.1
C22:6ω3	12.3	14.4
PUFA/SFA	2.1	1.8
DHA/EPA	0.8	0.9
C18:4ω3/C16:1ω7	0.6	0.4
C18:1ω9C/C18:1ω7C	2.7	2.7
18PUFA/16PUFA	12.8	11.8

There was no remarkable difference between *E. superba* adults and subadults for what concerns PUFAs, except that C22:6ω3 is higher in subadults.

Comparison of the FA results of the two species by non-parametric Mann–Whitney test failed to highlight significant differences for the whole set of lipid classes. This suggests that *E. superba* and *E. crystallorophias* differ less in the level of simple FAs than in how they form complex lipids, specifically triacylglycerols in *E. superba* and wax esters in *E. crystallorophias* [14], and in lipid body content. In any case, both species showed relatively high contents in FA classes as 22:6ω3 and 20:5ω3 and intermediate levels of 18:4ω3 and 16:1ω7, which indicate the presence of phytoplankton in the diet; moreover, they also presented a high level of FA class 18:1ω9C, plus low levels of 20:1, which denote feeding on zooplankton, even if at a more minor extent than on phytoplankton. On the bases of the specific FA ratios analysed, *E. crystallorophias* shows a more pronounced omnivorous diet in respect to *E. superba*, at least in austral summer, i.e., the season of the acoustic survey.

4. Discussion

4.1. Biochemical Composition of E. superba and E. crystallorophias

A predominance of protein has previously been described in marine benthic and planktonic organisms [27,42,43]. Herbivorous zooplankton species generally show high lipid levels [19,44]. High protein and low carbohydrate content have consistently been reported in most studies of marine crustaceans such as copepods [45], mysidaceans [46], harpacticoids [47], euphausiids [43], and amphipods [27]. Biochemical composition, particularly protein and lipid content, is held to be affected by the bioenergetics strategies adopted by populations inhabiting different environments [48]. Lipid accumulation is the most common long-term energy storage strategy in aquatic organisms, whose reproductive potential is largely determined by lipid content [41]. In this study, lipid concentrations were much higher in *E. crystallorophias* (Figure 2); this is probably due to the lower level of winter

consumption of lipid stores by ice krill, so that this species could be active in reproduction earlier in spring compared with *E. superba*, as hypothesized by Hagen and Auel [14].

Concerning the potential differences between different sizes in the same species in terms of biochemical composition, only one relationship could be detected through the analysis of species by size classes, which is a decrease in carbohydrate content with increasing size of *E. crystallorophias*. To our knowledge, there are no indications from literature on this specific aspect; the authors think that one possible explanation may be greater sugar consumption by adults due to different metabolic rates.

The present data clearly document that biochemical composition is affected primarily by species and secondarily by geographical factors, with latitude playing a greater role than distance from the coast. The hauls in the Southern Ocean or on its border with the Ross Sea (n. 1, 26, 27, 28, and 30) were quite close to each other and provided very similar data for *E. superba*, whereas haul n. 10, which is further south in the mideastern Ross Sea, yielded different data. Likewise, the biochemical composition of *E. crystallorophias* specimens was quite similar in the inshore hauls (n. 17, 13, and 8), and these differed from the one found in the offshore hauls (n. 7 and 10).

4.2. Fatty Acid Composition in E. superba and E. crystallorophias

Concerning fatty acid composition, the high content in 18:4ω3 and 22:6ω3 detected in both species seems to reflect a non-negligible number of dinoflagellates in their diets, whereas 16:1ω7, 20:5ω3, 14:0, 20:1, and 22:1 would reflect diatom and copepod consumption [22]. Krill samples examined in this work seem to be in an intermediate position between a spring-kind diet as reported in Ericson et al. [49], more oriented on diatoms and copepods, and a summer-kind diet, more based on flagellates, at least concerning *E. superba*. The period in which the acoustic survey that provided our samples was conducted could support these findings.

The difference between our data and those of Ju and Harvey [23], already mentioned in the 'Results' paragraph, is probably due to the sampling season, respectively austral summer (greater phytoplankton availability due to ice melting) and austral winter. Phleger et al. [50] and Ju and Harvey [23] performed analyses at various trophic levels. According to their data the major components of seston are 16:0, 18:0, 18:1ω9C, 18:1ω7, and 22:6ω3; those of ice-associated algae are 14:0, 16:0, 16:1ω7, 16:4ω1, and 20:5ω3; those found in the stomach contents of *E. superba* are 14:0, 16:0, 16:1ω7, 18:1ω9C, and 20:5ω3; and those found in copepods are 16:1ω7, 18:1ω9C, and 20:1.

A comparison with other data acquired in summer has been made with the findings of Yang et al. [51] and Ko et al. [52]; they both identify a higher omnivorous attitude in *E. crystallorophias* than in *E. superba*, basing their analysis on the PUFA/SFA, C18:1ω9C/C18:1ω7C, and 18PUFA/16PUFA ratios. Even in our case, PUFA/SFA and 18PUFA/16PUFA (see Table 4) are higher in *E. crystallorophias*, while there is no evident difference in C18:1ω9C/C18:1ω7C ratio between the two species. Two ratios out of three seem to indicate a more omnivorous diet for ice krill, so this conclusion could be valid also in our case because it is not only based on the PUFA/SFA ratio, which may be dependent on the total lipid content [53–55]; in fact, in our case *E. crystallorophias* presents higher lipid content at the same time as higher values of PUFA/SFA ratio (compare Table 3 and Figure 3). This result is not necessarily in contrast with the positive correlation between ice krill biomass and fluorescence as proxy of phytoplankton abundance found by Leonori et al. [2] in the same area, since the higher local concentration of phytoplankton may be associated with higher abundance of zooplankton organisms such as protists, ciliates, and small copepods [52] that could be in certain cases the main prey for adult ice krill.

No significant differences between *E. superba* adults and subadults were found for what concerns PUFAs; similar results were found by Cripps et al. [56], but in that case juveniles (not available in our samples) were found to have higher contents of PUFAs, meaning that they probably fed more on copepods than adults, also given the low local algal biomass. Interestingly, age class 0 Antarctic krill from the Northern Weddell Sea [16] presented high

concentrations of 16:0, 20:5ω3 (EPA), and 22:6ω3 (DHA), which are basic components of phospholipids; in this study, the same fatty acid classes were the most representative, plus C18:1ω9C and, to a minor extent, C18:1ω7C. Both *E. superba* and *E. crystallorophias* from the Weddell Sea [15] showed a very similar composition of fatty acid classes to those found for the Ross Sea samples analysed in the present paper, especially considering polar lipids, while some difference was evidenced for EPA and DHA for triacylglycerols, where these last two compounds were important only in certain seasons. These results added evidence regarding how biochemical composition in terms of fatty acids for the two krill species studied in this work are quite similar even in different Antarctic sectors.

Comparison of the FA results of the two species by non-parametric Mann–Whitney test failed to highlight significant differences for the whole set of lipid classes. This suggests that *E. superba* and *E. crystallorophias* differ less at the level of simple FAs than in how they form complex lipids, specifically triacylglycerols in *E. superba* and wax esters in *E. crystallorophias* [14], and in lipid body content. In any case, both species showed relatively high contents in FA classes as 22:6ω3 and 20:5ω3 and intermediate levels of 18:4ω3 and 16:1ω7, which indicate the presence of phytoplankton in the diet; moreover, they also presented a high level of FA class 18:1ω9C, plus low levels of 20:1, which denote feeding on zooplankton, even if at a more minor extent than on phytoplankton. On the bases of the specific FA ratios analysed, *E. crystallorophias* shows a more pronounced omnivorous kind of diet in respect to *E. superba*, at least in austral summer, i.e., the season of our survey.

In summary, the present work gave some interesting insights into the diet of the two krill species predominant in the Ross Sea, confirming their ability to switch their diets according to the surrounding environmental conditions and food availability. In particular, fatty acid composition presented an important presence of markers identifying diatoms and flagellates, but also copepods, evidencing krill adaptability to prey, also including zooplankton organisms and not exclusively phytoplankton that could be scarce in certain periods of the year, such as austral winter.

5. Conclusions

Both in *Euphausia superba* and *Euphausia crystallorophias* proteins were the predominant biochemical class of organic compounds, followed by lipids in *E. crystallorophias* and carbohydrates in *E. superba*. The main differences in terms of biochemical compositions were found between the two species; secondary in importance were differences in terms of spatial localization (latitude, distance from the coast).

Fatty acid analysis denoted a prevalent herbivorous kind of diet for both species, even with a minor presence of zooplankton. PUFA/SFA and 18PUFA/16PUFA ratios were higher in *E. crystallorophias*, meaning that this species presents a more omnivorous kind of diet in respect to *E. superba*, at least in austral summer. Comparison of the fatty acid classes of the two species did not highlight significant differences for the whole set of lipid classes, suggesting that *E. superba* and *E. crystallorophias* differ less at the level of simple FAs than in how they form complex lipids, specifically triacylglycerols in *E. superba* and wax esters in *E. crystallorophias*.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d15040480/s1, Table S1: (a) Biochemical composition of individuals of the two species collected at haul n. 10 analysed with one-way ANOVA and Newman–Keuls test; (b) differences among hauls and among 2 mm TL size classes in *E. superba* and *E. crystallorophias* analysed using non-parametric Kruskal–Wallis one-way ANOVA. ns = not significant, TL = total length; Table S2: Biochemical composition of the two krill species analysed using Mann–Whitney's test; Table S3: Similarity percentages among samples from the same haul and dissimilarity percentage among hauls according to different parameters; Table S4: Hauls displaying significant differences ($p < 0.1\%$). 10s = *E. superba* from haul n. 10, 10c = *E. crystallorophias* from haul n. 10.

Author Contributions: Conceptualization, A.D.F. and E.M.; methodology, A.D.F. and E.M.; software, A.D.F., E.M., and I.L.; validation, A.D.F., E.M., I.B., and I.L.; formal analysis, A.D.F., E.M., and I.L.; investigation, A.D.F. and E.M.; resources, A.D.F., E.M., and I.L.; data curation, A.D.F.; writing—original draft preparation, A.D.F.; writing—review and editing, A.D.F., E.M., I.B., and I.L.; visualization, A.D.F., E.M., I.B., and I.L.; supervision, E.M. and I.L.; project administration, I.L.; funding acquisition, I.L. All authors have read and agreed to the published version of the manuscript.

Funding: Authors are grateful to the Italian National Antarctic Scientific Commission (CSNA) and the Italian National Research Program in Antarctica (PNRA) for financial support: research project 2002/8.4 (KEP).

Institutional Review Board Statement: The animal study protocol was approved by the Antarctic Technical Unit—Unit of Project Research, Technological Innovation, and Environmental Protection (UTA-RIA) of the National Agency for new Technologies, Energy, and Sustainable Economic Development (protocol code ENEA/2021/62121/UTA, 13 October 2021; antecedent analogous permits without protocol code were issued on 12 November 2013 and 23 January 2016).

Informed Consent Statement: Not applicable.

Data Availability Statement: The data of the present paper are available on request.

Acknowledgments: The authors would like to thank the Italian National Research Program in Antarctica (PNRA). We also wish to express our gratitude to the crew of *R/V Italica* and all the researchers involved for their assistance in conducting the scientific surveys. We'd like to thank Roberto Danovaro (Università Politecnica delle Marche) for the access to laboratory facilities and assistance for biochemical analyses and Natale G. Frega and Paolo Lucci (Università Politecnica delle Marche) for access to laboratory facilities and for their assistance for fatty acid analysis.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Azzali, M.; Kalinowski, J. Spatial and temporal distribution of krill *Euphausia superba* biomass in the Ross Sea (1989–1990 and 1994). In *Ross Sea Ecology*; Faranda, F., Guglielmo, L., Ianora, A., Eds.; Springer: Berlin/Heidelberg, Germany; New York, NY, USA, 2000; pp. 433–455.
2. Leonori, I.; De Felice, A.; Canduci, G.; Costantini, I.; Biagiotti, I.; Giuliani, G.; Budillon, G. Krill distribution in relation to environmental parameters in mesoscale structures in the Ross Sea. *J. Mar. Sys.* **2017**, *166*, 159–171. [CrossRef]
3. Murphy, E.J.; Cavanagh, R.D.; Hofmann, E.E.; Hill, S.L.; Constable, A.J.; Costa, D.P.; Pinkerton, M.H.; Johnston, N.M.; Trathan, P.N.; Klinck, J.M.; et al. Developing integrated models of Southern Ocean food webs: Including ecological complexity, accounting for uncertainty and the importance of scale. *Prog. Ocean.* **2012**, *102*, 74–92. [CrossRef]
4. Hewitt, R.P.; Lipsky, J.D. Krill and other plankton. In *Encyclopedia of Marine Mammals*, 3rd ed.; Academic Press: Cambridge, MA, USA, 2018; pp. 537–543.
5. Cavan, E.L.; Belcher, A.; Atkinson, A.; Hill, S.L.; Kawaguchi, S.; McCormack, S.; Meyer, B.; Nicol, S.; Ratnarajah, L.; Schmidt, K.; et al. The importance of Antarctic krill in biogeochemical cycles. *Nat. Comm.* **2019**, *10*, 4742. [CrossRef]
6. Nicol, S. *Antarctic Krill Sustainability*; Aker Bio Marine Antarctic: Lysaker, Norway, 2014.
7. Quetin, L.B.; Ross, R.M. Environmental variability and its impact on the reproductive cycle of Antarctic krill. *Amer. Zool.* **2001**, *41*, 74–89. [CrossRef]
8. Rakusa-Suszczewski, S.; Zdanowski, M.K. Bacteria in krill (*Euphausia superba Dana*) stomach. *Acta Protozool.* **1989**, *28*, 87–90.
9. Flores, H.; Atkinson, A.; Kawaguchi, K.; Krafft, B.A.; Milinevsky, G.; Nicol, S.; Reiss, C.; Tarling, G.A.; Werner, R.; Bravo Rebolledo, E.; et al. Impact of climate change on Antarctic krill. *Mar. Ecol. Prog. Ser.* **2012**, *458*, 1–19. [CrossRef]
10. Veytia, D.; Corney, S.; Meiners, K.M.; Kawaguchi, S.; Murphy, E.J.; Bestley, S. Circumpolar projections of Antarctic krill growth potential. *Nat. Clim. Change* **2020**, *10*, 568–575. [CrossRef]
11. Perissinotto, R.; Gurney, L.; Pakhomov, E.A. Contribution of heterotrophic material to diet and energy budget of Antarctic krill, *Euphausia superba*. *Mar. Biol.* **2000**, *136*, 129–135. [CrossRef]
12. Schmidt, K.; Atkinson, A.; Petzke, K.; Voss, M.; Pond, D.W. Protozoans as a food source for Antarctic krill, *Euphausia superba*: Complementary insights from stomach content, fatty acids, and stable isotopes. *Limn. Ocean.* **2006**, *51*, 2409–2427. [CrossRef]
13. Ikeda, T.; Dixon, P. Observations on moulting in Antarctic krill (*Euphausia superba Dana*). *Aust. J. Mar. Freshw. Res.* **1982**, *33*, 71–76. [CrossRef]
14. Hagen, W.; Auel, H. Seasonal adaptations and the role of lipids in oceanic zooplankton. *Zoology* **2001**, *104*, 313–326. [CrossRef]
15. Falk-Petersen, S.; Hagen, W.; Kattner, G.; Clarke, A.; Sargent, J. Lipids, trophic relationships, and biodiversity in Arctic and Antarctic krill. *Can. J. Fish. Aquat. Sci.* **2000**, *57*, 178–191. [CrossRef]

16. Schaafsma, F.L.; Kohlbach, D.; David, C.; Lange, B.A.; Graeve, M.; Flores, H.; van Franeker, J.A. Spatio-temporal variability in the winter diet of larval and juvenile Antarctic krill, *Euphausia superba*, in ice-covered waters. *Mar. Ecol. Prog. Ser.* **2017**, *580*, 101–115. [CrossRef]
17. Hagen, W.; Van Vleet, E.S.; Kattner, G. Seasonal storage as overwintering strategy of Antarctic krill. *Mar. Ecol. Prog. Ser.* **1996**, *134*, 85–89. [CrossRef]
18. Nicol, S.; Virtue, P.; King, R.; Davenport, S.R.; McGaffin, A.F.; Nichols, P. Condition of *Euphausia crystallorophias* off East Antarctica in winter in comparison to other seasons. *Deep. Sea Res. Part II Top. Stud. Oceanogr.* **2004**, *51*, 2215–2224. [CrossRef]
19. Lee, R.F.; Hagen, W.; Kattner, G. Lipid storage in marine zooplankton. *Mar. Ecol. Prog. Ser.* **2006**, *307*, 273–306. [CrossRef]
20. Pond, D.W.; Tarling, G.A. Phase transitions of wax esters adjust buoyancy in diapausing *Calanoides acutus*. *Limn. Ocean.* **2011**, *56*, 1310–1318. [CrossRef]
21. Pond, D.W. The physical properties of lipids and their role in controlling the distribution of zooplankton in the oceans. *J. Plank. Res.* **2012**, *34*, 443–453. [CrossRef]
22. Hellessey, N.; Ericson, J.A.; Nichols, P.D.; Kawaguchi, S.; Nicol, S.; Hoem, N.; Virtue, P. Regional diet in Antarctic krill (*Euphausia superba*) as determined by lipid, fatty acid, and sterol composition. *Polar Biol.* **2022**, *45*, 1077–1092. [CrossRef]
23. Ju, S.J.; Harvey, H.R. Lipids as markers of nutritional condition and diet in the Antarctic krill *Euphausia superba* and *Euphausia crystallorophias* during austral winter. *Deep-Sea Res. II* **2004**, *51*, 2199–2214. [CrossRef]
24. De Felice, A.; Biagiotti, I.; Canduci, G.; Costantini, I.; Malavolti, S.; Giuliani, G.; Leonori, I. Is It the Same Every Summer for the Euphausiids of the Ross Sea? *Diversity* **2022**, *14*, 433. [CrossRef]
25. Azzali, M.; Russo, A.; Sala, A.; De Felice, A.; Catalano, B. Preliminary results of a survey on krill, environment and predators in CCAMLR Division 88.1 carried out in December 2003 and in January 2004 (Project 8.4). In Proceedings of the CCAMLR WG-EMM-04/71, Siena, Italy, 12–23 July 2004.
26. Hartree, E.F. Determination of protein: A modification of the Lowry method that gives a linear photometric response. *Anal. Biochem.* **1972**, *48*, 422–427. [CrossRef] [PubMed]
27. Perrone, F.M.; Della Croce, N.; Dell'Anno, A. Biochemical composition and trophic strategies of the amphipod *Eurythenes grillus* at hadal depths (Atacama Trench, South Pacific). *Chem. Ecol.* **2003**, *19*, 441–449. [CrossRef]
28. Lowry, O.H.; Rosebrough, N.J.; Farr, A.L.; Randall, R.J. Protein measurement with the Folin-Phenol reagents. *J. Biol. Chem.* **1951**, *193*, 265–275. [CrossRef]
29. Dubois, N.; Gilles, K.A.; Hamilton, J.K.; Rebers, P.A.; Smith, F. Colorimetric method for determination of sugars and related substances. *Analyt. Chem.* **1956**, *28*, 350–356. [CrossRef]
30. Gerchakov, S.M.; Hatcher, P.G. Improved technique for analysis of carbohydrates in sediments. *Limn. Ocean* **1972**, *17*, 938–943. [CrossRef]
31. Bligh, E.G.; Dyer, W.J. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **1959**, *37*, 911–917. [CrossRef]
32. Folch, J.; Lees, M.; Sloane-Stanley, G.H. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **1957**, *226*, 497–509. [CrossRef]
33. Christie, W.W. Preparation of derivatives of fatty acids. In *Lipid Analysis*, 3rd ed.; The Oily Press: Bridgwater, UK, 2003; p. 416.
34. De Felice, A. Stock e Bioenergetica di due Specie di Eufausiacei (*E. superba* ed *E. crystallorophias*) nel Mare di Ross. Ph.D. Thesis, Università Politecnica delle Marche, Ancona, Italy, 9 February 2010.
35. Sala, A.; Azzali, M.; Russo, A. Krill of the Ross Sea: Distribution, abundance and demography of *Euphausia superba* and *Euphausia crystallorophias* during the Italian Antarctic Expedition (January–February 2000). *Sci. Mar.* **2002**, *66*, 123–133. [CrossRef]
36. Watkins, J.L. Variations in the size of Antarctic krill, *Euphausia superba Dana*, in small swarms. *Mar. Ecol. Prog. Ser.* **1986**, *31*, 67–73. [CrossRef]
37. Azzali, M.; Leonori, I.; De Felice, A.; Russo, A. Spatial-temporal relationships between two euphausiid species in the Ross Sea. *Chem. Ecol.* **2006**, *22*, S219–S233. [CrossRef]
38. Murase, H.; Kitakado, T.; Hakamada, T.; Matsuoka, K.; Nishiwaki, S.; Naganobu, M. Spatial distribution of Antarctic minke whales (*Balaenoptera bonaerensis*) in relation to spatial distributions of krill in the Ross Sea, Antarctica. *Fish. Ocean* **2013**, *22*, 154–173. [CrossRef]
39. Sologub, D.O.; Remeslo, A.V. Distribution and size-age composition of Antarctic krill (*Euphausia superba*) in the South Orkney Islands region (CCAMLR subarea 48.2). *CCAMLR Sci.* **2011**, *18*, 123–134.
40. Azzali, M.; Leonori, I.; Lanciani, G. A hybrid approach to acoustical classification and length estimation of krill. *CCAMLR Sci.* **2004**, *11*, 33–58.
41. Sargent, J.R.; Henderson, R.J. Lipids. In *The Biological Chemistry of Marine Copepods*; Corner, E.D.S., O'Hara, S.C.M., Eds.; Clarendon Press: Oxford, UK, 1986; pp. 59–108.
42. Danovaro, R.; Pusceddu, A.; Covazzi Harriague, A.; Marrale, D.; Dell'Anno, A.; Petrillo, M.; Albertelli, G.; Della Croce, N. Community experiments using benthic chambers: Microbial significance in highly organic enriched sediments. *Chem. Ecol.* **1999**, *16*, 7–30. [CrossRef]
43. Virtue, P.; Johannes, R.E.; Nichols, P.D.; Young, J.W. Biochemical composition of *Nyctiphanes australia* and its possible use as an aquaculture feed source: Lipids, pigments and fluoride content. *Mar. Biol.* **1995**, *122*, 121–128. [CrossRef]
44. Clarke, A.; Peck, L.S. The physiology of polar marine zooplankton. *Polar Res.* **1991**, *10*, 355–369. [CrossRef]

45. Bamstedt, U. Studies on the deep-water pelagic community of Korsfjorden, western Norway. Ecological aspects of individual variations in weight and protein and lipid content of *Euchaeta norvegica* (Copepoda). *Sarsia* **1976**, *61*, 15–30.
46. Bamstedt, U. Studies on the deep-water pelagic community of Korsfjorden, western Norway. Seasonal variation in weight and biochemical composition of *Chiridius armatus* (Copepoda), *Boreomysis arctica* (Mysidacea), and *Eukrohnia hamata* (Chaetognata) in relation to their biology. *Sarsia* **1978**, *63*, 145–154. [CrossRef]
47. Miliou, H.; Moraitou-Apostolopoulou, M. Effects of seven diets on the population dynamics of laboratory cultured *Tisbeholothuriae Humes* (Copepoda, Harpacticoida). *Helgol. Meeresunters* **1991**, *45*, 345–356. [CrossRef]
48. Lehtonen, K.K. Ecophysiology of the benthic amphipod *Monoporeia affinis* in an open-sea area of the northern Baltic Sea: Seasonal and interannual variations in body composition, with bioenergetic considerations. *Mar. Ecol. Prog. Ser.* **1996**, *14*, 87–98. [CrossRef]
49. Ericson, J.A.; Hellessey, N.; Nichols, P.D.; Nicol, S.; Kawaguchi, S.; Hoem, N.; Virtue, P. New insights into the seasonal diet of Antarctic krill using triacylglycerol and phospholipid fatty acids, and sterol composition. *Pol. Biol.* **2019**, *42*, 1985–1996. [CrossRef]
50. Phleger, C.F.; Nelson, M.M.; Mooney, B.D.; Nichols, P.D. Interannual and between species comparison of the lipids, fatty acids and sterols of Antarctic krill from the US AMLR Elephant Island survey area. *Comp. Biochem. Physiol. Part B* **2002**, *131*, 733–747. [CrossRef] [PubMed]
51. Yang, G.; Li, C.; Wang, Y. Fatty acid composition of *Euphausia superba*, *Thysanoessa macrura* and *Euphausia crystallorophias* collected from Pridz Bay, Antarctica. *J. Ocean. Univ. China* **2016**, *15*, 297–302. [CrossRef]
52. Ko, A.R.; Yang, E.J.; Kim, M.S.; Ju, S.-J. Trophodynamics of euphausiids in the Amundsen Sea during the austral summer by fatty acid and stable isotopic signatures. *Deep-Sea Res. II* **2016**, *123*, 78–85. [CrossRef]
53. Hagen, W.; Kattner, G.; Terbruggen, A.; Van Vleet, E.S. Lipid metabolism of the Antarctic krill *Euphausia superba* and its ecological implications. *Mar. Biol.* **2001**, *139*, 95–104. [CrossRef]
54. Kattner, G.; Hagen, W. Lipid metabolism of the Antarctic euphausiid *Euphausia crystallorophias* and its ecological implication. *Mar. Ecol. Prog. Ser.* **1998**, *170*, 203–213. [CrossRef]
55. Stübing, D.; Hagen, W. Fatty acid biomarker ratios-suitable trophic indicators in Antarctic euphausiids? *Pol. Biol.* **2003**, *26*, 774–782. [CrossRef]
56. Cripps, G.C.; Watkins, J.L.; Hill, H.J.; Atkinson, A. Fatty acid content of Antarctic krill *Euphausia superba* at South Georgia related to regional populations and variations in diet. *Mar. Ecol. Prog. Ser.* **1999**, *181*, 177–188. [CrossRef]