Vol. 266: 43–58, 2004 | MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser | Published January 30

Trophic-level interpretation based on $\delta^{15}N$ values: implications of tissue-specific fractionation and amino acid composition

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ABSTRACT: Stable nitrogen isotope ratios are routinely used to disentangle trophic relationships. Several authors have discussed factors in addition to diet that might contribute to variability in $\delta^{15}N$ of consumers, but few studies have explored such factors in detail. For a better understanding of tissue-specific differences in $\delta^{15}N$, we examined postlarval euphausiids across a variety of seasons and regions in the Southern Ocean. The concentration and $\delta^{15}N$ of individual amino acids were analysed to account for both the biochemical and physiological underpinnings of the observed bulk $\delta^{15}N$. Euphausiids showed consistent $d^{15}N$ differences of 1 to 2 ‰ between the digestive gland and abdominal segment, and between reproductively active males and females. These differences in bulk $\delta^{15}N$ were accompanied by variations in relative proportions of amino acids (up to 5 mol %) and their $\delta^{15}N$ (up to 11‰). Aspartic acid and glutamic acid had the strongest influence on bulk $\delta^{15}N$, due to their high abundance and variable $\delta^{15}N$ values. Differences in relative proportions and/or $\delta^{15}N$ of glycine and alanine were also important for bulk $\delta^{15}N$ values. Isotopic variations in amino acids between gender and tissues were explained by dominant internal processes such as protein synthesis or degradation for energy supply, and by differences in amino acid pool sizes. Despite the offset in bulk $\delta^{15}N$ between females and males, several lines of evidence suggested that their trophic levels were similar. Thus, specific amino acid composition and metabolism may confound trophic level interpretations of bulk $\delta^{15}N$ values. Micronekton are normally analyzed whole in isotopic studies, and we suggest that their analyses should be restricted to comparable tissues such as muscles.

KEY WORDS: Stable isotopes · $\delta^{15}N$ · Amino acids · Tissue · *Euphausia superba* · Male · Female

INTRODUCTION

The analysis of naturally occurring stable nitrogen and carbon isotope ratios is an important tool in trophic ecology. The general assumption of the approach is a predictable relationship between the isotopic composition of a consumer and its food source. Bulk carbon is transferred rather conservatively, while bulk nitrogen is fractionated, with ^{15}N becoming enriched by ca. 3 to 4‰ from one trophic level to the next (Michener & Schell 1994). Thus, carbon isotope ratios can trace different sources of primary productivity, whereas nitrogen isotope ratios are used as trophic position indicators. Stable isotope approaches have improved our understanding of food-web relationships, resource partitioning, the importance of specific nutrient sources and habitat usage of migrating species (Peterson & Fry 1987, Kiyashko et al. 2001, Lesage et al. 2001).

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However, interpretation of isotope ratios is not always straightforward and can lead to erroneous conclusions (Gannes et al. 1997). Problems are partly caused by ecosystem complexities such as multiple food sources or variable growth and turnover rates in relation to an isotopically changing food-web baseline (O'Reilly et al. 2002, Schmidt et al. 2003). There are, however, further problems related to the fundamental principles of the method. Isotopic fractionation is not constant but can vary depending on food quality (Fantle et al. 1999, Adams & Sterner 2000) and nutritional stress (Hobson et al. 1993). Even with a single, isotopically consistent food source, species can show different isotope values (DeNiro & Epstein 1981, Macko et al. 1982) and so do various tissues from the same individual (Tieszen et al. 1983, Hobson & Clark 1992a).

Consumer size has largely dictated the sampling protocol for isotope analyses. Studies on zooplankton and micronekton are usually based on whole-body samples, whereas for larger consumers like fish, birds and mammals, individual components are analysed (Schell et al. 1989, Hobson & Welch 1992, Lesage et al. 2001). Measuring several body parts allows short-, intermediate- and long-term dietary information to be compared, as tissues vary in their turnover rates and thus in their periods of food source integration (Tieszen et al. 1983, Hobson & Clark 1992b, Kurle 2002). However, tissues might also vary in their trophic fractionation. Even when equilibrated with the diet, muscle and liver of the same individual can differ by 0.5 to 1.5‰ in $\delta^{15}N$ and $\delta^{13}C$ (DeNiro & Epstein 1981, Tieszen et al. 1983, Hobson & Clark 1992a, Hobson et al. 1996, Pinnegar & Polunin 1999). Differences between other tissues, such as liver and hair or pancreas and brain, are up to 3‰ (DeNiro & Epstein 1978, 1981, Tieszen et al. 1983). This variation is a caveat not only to trophic interpretations in larger species, e.g. when comparisons are based on $\delta^{15}N$ of different tissues or when the relative importance of a food source is calculated (Tieszen et al. 1983, Hobson & Clark 1992a, Pinnegar & Polunin 1999), but it also applies to smaller species analysed whole, if their biochemical composition differs.

Tissue-specific patterns in $\delta^{13}C$ and $\delta^{15}N$ are often attributed to their biochemical compositions (Hobson & Clark 1992a, Kurle 2002). For example, a lipid-rich tissue would have a lower $\delta^{13}C$ than a protein-rich tissue, since lipids are depleted in ^{13}C relative to proteins (Tieszen et al. 1983). The amino acid composition of a tissue can also influence its isotope values. $\delta^{13}C$ and $\delta^{15}N$ values of individual amino acids cover a wide range (Macko et al. 1987, Hare et al. 1991, McClelland & Montoya 2002), and tissues vary in their common proteins, and therefore, in their amino acid composition (Wilson & Poe 1985, Gunasekera et al. 1997). Collagen, for example, has a high $\delta^{13}C$ because the most common amino acid, glycine, is enriched in ^{13}C compared to other amino acids (Hare et al. 1991). While differences in biochemical compositions of tissues are one source of variability in isotope values, a given amino acid might also be fractionated differently according to the metabolic state of the animal. Hobson et al. (1993) found significant ^{15}N enrichment in the bone collagen of fasting birds compared to well-fed controls, even though amino acid profiles differed only slightly. They suggested that additional isotopic fractionation associated with catabolism, protein mobilization, or redeposition was important. However, processes that lead to fractionation in different tissues are still poorly understood (Gannes et al. 1997). Thus, effective use of stable isotope composition for dietary reconstructions requires an awareness of the basic physiological processes (Gannes et al. 1997).

The target species of the present study is Antarctic krill *Euphausia superba*, which has a central role in the Southern Ocean food web (Hopkins et al. 1993). Isotopic studies on krill, as on other zooplankton and micronekton species, have so far been based on whole-animal analysis (e.g. Wada et al. 1987, Rau et al. 1991), and none has yet analysed the nitrogen isotope values of the component amino acids, which largely determine the bulk $\delta^{15}N$. The aim of our study was to examine tissue-specific variability in $\delta^{15}N$ of krill and to shed light on factors that lead to these differences. First, we analysed $\delta^{15}N$ in digestive gland region, abdominal segment and remaining body over a number of seasons and stations to evaluate the range and consistency in isotopic differences between tissues. Second, the relative proportion and $\delta^{15}N$ of individual amino acids were analysed from tissues of reproductively active males and gravid females. Such compositional and isotopic measurements have rarely been combined, but are essential to understand both the biochemical and physiological underpinnings for stable isotope ratios of whole organisms and specific tissues.

MATERIALS AND METHODS

Field sampling. ***South Georgia, January 1996:*** Juvenile and adult *Euphausia superba* were caught north of South Georgia by oblique hauls of a Rectangular Midwater Trawl deployed to a depth of 250 m (Cripps et al. 1999). Samples were immediately stored at –80°C. Krill were analysed from Stn B2 (53.84° S, 38.96° W), and sampled on 26 January 1996 (Fig. 1 in Cripps et al. 1999).

Polar Front and Lazarev Sea, April to May 1999: Immature postlarval *Euphausia frigida* and *Thysanoessa* spp. were collected in the Polar Front (~49° S, 20° E) and *E. superba* in the south-western Lazarev

Sea (~69° S, 5° W) during April and May 1999. Samples were taken from the top 150 m of the water column using vertical tows of a Bongo net. Individuals were sorted immediately under a stereomicroscope and transferred to filtered seawater to evacuate their guts. After about 24 h, krill were filtered onto a mesh and stored at −80°C.

South Shetland Islands, February-March 2000: Adult *Euphausia superba* were collected near the South Shetland Islands by oblique tows of an Isaac-Kidd Midwater Trawl through the upper 170 m of the water column. Krill were sampled from 3 stations: Stn A, 61° 30′ S, 59° W on 25 February; Stn B, 62° S, 60° W on 24 February; and Stn C, 60° 45′ S, 54° 30′ W on 4 March. Animals were kept in filtered seawater for ca. 24 h to evacuate their guts. Non-gravid females, gravid females and reproductively active males were identified according to Makarov & Denys (1981).

Sample preparation. Frozen euphausiids were dissected into 3 parts: digestive gland region, abdominal segment 3 and the remaining body (Fig. 1). The exoskeleton was removed from the digestive gland region and abdominal segment 3. Each of the 3 parts was freeze-dried, weighed and ground in an agate mortar. The digestive gland region accounted for 9.2 ± 2.5% of the body dry weight, abdominal segment 3 for 8.1 ± 1.9% and the remaining body for 80.4 ± 2.4%. The $\delta^{15}N$ and $\delta^{13}C$ values of the whole body were recalculated according to mass/isotope balance equations using the isotope values of digestive gland region, abdominal segment 3 and remaining body, and their proportion to the total body nitrogen.

Analyses. The bulk $\delta^{15}N$ and $\delta^{13}C$ were measured for each euphausiids individually. Thereafter, tissues of 9 reproductively active males and 9 gravid females, sampled near the South Shetland Islands in March 2000, were pooled to analyse composition and $\delta^{15}N$ of amino acids.

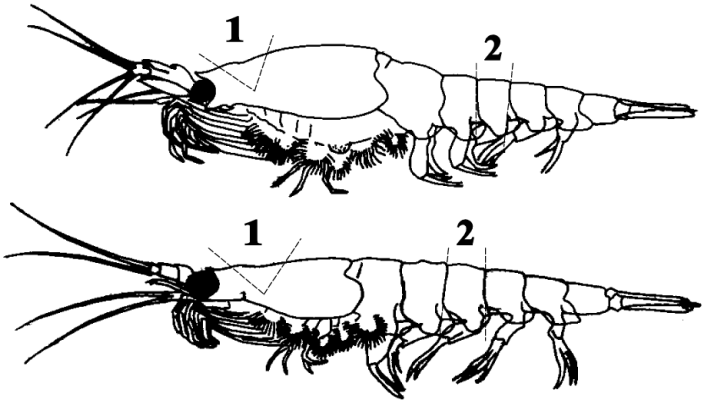

Fig. 1. *Euphausia superba*. Dissection of gravid female (top) and male (bottom) into 3 body fractions: digestive gland region (1), abdominal segment 3 (2), and the remaining body

Bulk nitrogen and carbon isotope analysis: Carbon and nitrogen stable isotope ratios were analysed using a CHN analyser (Thermofinnigan CE 1108) combined with a mass spectrometer (Finnigan Delta S) via a Conflow II open split interface. Calibration for the total carbon and nitrogen determination was done daily with an acetanilide standard. All isotope abundances are expressed in δ notation as follows: δX (‰) = $[(R_{sample} / R_{standard}) - 1] \times 10^3$, where X is ^{13}C or ^{15}N, and R is the $^{13}C:^{12}C$ or $^{15}N:^{14}N$ ratio. PeeDee Belemnite carbonate (NBS 21 and 22) and atmospheric nitrogen (IAEA-N1,-N2,-N3) were used as the standards for carbon and nitrogen, respectively. A laboratory internal standard (Peptone, Merck) was run for every 6th sample. The peptone standard indicated an analytical error associated with the isotope measurements of less than ±0.2‰ for both isotopes. Two to 3 replicates were analysed from each sample of ground krill tissue.

The $\delta^{13}C$ values of male and female *Euphausia superba* were corrected for variable lipid content according to Schmidt et al. (2003). In brief, the relative proportion of lipids was calculated from C:N ratios [lipid content (%) = 8.5301(C:N ratio) − 23.099], and lipids were assumed to be depleted in $\delta^{13}C$ relative to proteins by 6‰.

Amino acid composition: Both amino acid composition and $\delta^{15}N$ of individual amino acids were analysed from total (i.e. protein-bound plus free) amino acids. Samples of 50 mg dry mass were hydrolysed with 6 M hydrochloric acid (HCL) for 24 h at 110°C (Dall & Smith 1987, Gunasekera et al. 1997). A 100 µl subsample of the hydrolysate was dried under vacuum, redissolved in 100 ml 0.1 M HCL and purified by filtration through a 10 kDa cut-off filter. Amino acids were quantified using an Applied Biosystems 420A amino acid analyser. With this method, cysteine and tryptophan are only 20 to 30% recoverable and were therefore not included in the amino acid profile. Norleucin was used as an internal standard (0.01 N) to adjust for variation between samples. An external standard solution of 18 amino acids (Pierce Chemicals) was run for every 9th sample. In 9 consecutive standards, the coefficient of variation was equal to or less than 5% for all amino acids.

Nitrogen isotopic composition of amino acids: Samples were prepared for isotopic analysis of amino acids using the method of Metges et al. (1996). Five mg of dried sample was placed in a 16 × 100 mm glass tube with a polyetrafluoroethylene (PTFE)-lined cap and hydrolysed with ultra pure 6 M HCL for 24 h at 110°C. The hydrolysate was evaporated to dryness at 55°C under a stream of N_2. The residue was redissolved in 2 ml of 0.01 M HCl with

400 µl of 2.5 mM α-aminoadipic acid (internal standard) in 0.10 M HCl. This solution was then purified by filtration (0.65 µm Durapore filter) followed by cation exchange chromatography (Dowex 50WX8-400 ion exchange resin) in a 5 cm column prepared in a Pasteur pipette. Amino acids were eluted to dryness under a stream of N_2 at 80°C. Finally, the purified amino acids were derivatised to NPP-amino acid esters (a multi-step process) and dried down under a gentle stream of N_2 at room temperature. Dried residues were dissolved in 75 µl of ethyl acetate and stored in septum cap vials until analysis.

The stable isotopic composition of nitrogen in NPP derivatives of amino acids were analysed by gas chromatography/combustion/isotope ratio mass spectrometry (GC/C/IRMS) using a Micromass Isoprime mass spectrometer interfaced to a Hewlett Packard 6890 gas chromatograph. Mixes of amino acid derivatives from samples were injected into the GC, separated on an HP-Ultra 2 column (50 m × 0.32 mm i.d., 0.5 µm film thickness), combusted (850°C), reduced (500°C), and finally passed through a liquid nitrogen cold trap to remove water and CO_2 before entering the mass spectrometer. Nitrogen isotope ratios for each amino acid in a mix were measured sequentially.

Gas chromatography conditions were set to optimise peak separation and shape. Details of these conditions are given in McClelland & Montoya (2002). Each sample run was preceded by 2 pulses of reference N_2 and followed by 3 pulses of reference N_2, whose isotopic composition was calibrated against a variety of organic standards (peptone, histidine, and acetanilide) by continuous flow isotope ratio mass spectrometry (CFIRMS) using a Carlo Erba NA 2100 elemental analyser interfaced to a Micromass Optima mass spectrometer.

GC/C/IRMS analyses of standard mixtures of amino acids showed that values for aspartic acid, glutamic acid, proline, tyrosine, and valine were within 0.5‰ of the expected value; leucine, lysine, methionine and phenylalanine were within 1.0‰ of the expected value; and alanine, glysine, isoleucine, and threonine were within 1.5‰ of the expected value. The aggregate difference between measured and expected values for the amino acids listed above was 0.1 ± 0.8‰ (SD), confirming that no consistent bias was introduced by the derivatization and analysis procedure.

Statistics. A 1-sample Student's *t*-test was used to test the null hypothesis that there is no difference in $\delta^{15}N$ or $\delta^{13}C$ between either digestive gland and whole animal or abdominal segment and whole animal. Multiple comparisons of means were carried out with a Student-Newman-Keuls test. Differences were considered significant when $p < 0.05$.

RESULTS

Tissue- and gender-specific differences in bulk $\delta^{15}N$ and $\delta^{13}C$ of euphausiids

Euphausiids showed consistent differences in $\delta^{15}N$ and $\delta^{13}C$ values between body tissues across a variety of species, stages, regions and seasons (Table 1). The digestive gland region was isotopically lighter than the whole body, while the abdominal segment was heavier. In juveniles and immature adults, both tissues deviated from the whole body by ca. 1‰ in $\delta^{15}N$ and 1 to 2‰ in $\delta^{13}C$.

Gravid females and males of *Euphausia superba* sampled near South Georgia in summer 1996 and near the South Shetland Islands in summer 2000 showed differences in their $\delta^{15}N$ values of digestive gland and whole body similar to those of immature krill (Table 1). However, males differed little in $\delta^{15}N$ between abdominal segment and whole body, while differences for females exceeded that of immature krill. Thus, females were isotopically lighter than males in their digestive gland region and whole body ($p < 0.05$, Student-Newman-Keuls test), but not in their abdominal segment. At both sampling sites, there was a gender-specific difference in whole body $\delta^{15}N$ of 1.3 ‰, which is equivalent to nearly half a trophic level.

At the South Shetland Islands, differences between krill males and females were not restricted to $\delta^{15}N$, but were also observed for body mass, $\delta^{13}C$ and C:N ratios (Fig. 2). Variation between stations was minor. Gravid females were ca. ⅓ heavier than males, even though the total length was about the same (57 mm on average). Males had higher $\delta^{13}C$ values than females ($p < 0.05$, Student-Newman-Keuls test), but showed no variation between tissues. The $\delta^{13}C$ of females were similar in the digestive gland region and whole body samples, but significantly higher in the abdominal segment ($p < 0.05$, Student-Newman-Keuls test). The C:N ratios indicated lower proportions of lipids in the whole body and abdominal segment compared to the digestive gland region, and in males compared to females ($p < 0.05$, Student-Newman-Keuls test).

Since lipids are depleted in $\delta^{13}C$ relative to protein, C:N ratios were used to estimate the lipid content and thus the $\delta^{13}C$ values of male and female krill were corrected for variable proportions of lipids. Corrected $\delta^{13}C$ values were 0.5 to 2.0‰ higher than uncorrected values. However, the differences in $\delta^{13}C$ between males and females, and between various tissues of females remained even after this correction ($p < 0.05$, Student-Newman-Keuls test). The whole body of males and females differed by 3.2‰ in $\delta^{13}C$, and the lipid content explained only 0.8‰ of this difference.

Table 1. *Euphausia superba*, *E. frigida* and *Thysanoessa* spp. Tissue-specific stable isotope values of immature and mature krill sampled within different regions and seasons (SG: South Georgia, January 1996; PF: Polar Front, April to May 1999; LS: Lazarev Sea, April 1999; SI: South Shetland Is., March 2000). n: number of individuals measured as replicates. Mean dry weight, $\delta^{15}N$ and $\delta^{13}C$ of the whole body are given (±1 SD). Values of the digestive-gland region (digest. gland) and abdominal segment 3 (abd. segm.) are expressed as difference to the whole body (mean ± 1SD). Significant differences are given in *italic* ($p < 0.05$; 1-sample *t*-test). Gravid (gr.) and non-gravid (n-gr.) females were separated. Mean values are given for immature krill, males and females

Species/stage	Region	n	Dry weight (mg ind.$^{-1}$)	$\delta^{15}N$(‰) whole body	$\Delta\delta^{15}N$(‰) (digest. gland – whole body)	$\Delta\delta^{15}N$(‰) (abd. segm. – whole body)	$\delta^{13}C$(‰) whole body	$\Delta\delta^{13}C$(‰) (digest. gland – whole body)	$\Delta\delta^{13}C$(‰) (abd. segm. – whole body)
Immature									
E. superba juvenile	SG	20	126 ± 33	3.5 ± 0.6	*–1.0 ± 0.2*	*0.7 ± 0.1*	–26.3 ± 1.5	*–1.0 ± 0.5*	*1.3 ± 0.4*
Thysanoessa spp. juvenile	PF	3	30 ± 3	5.3 ± 0.9	–0.9 ± 0.4	0.7 ± 0.4	–22.2 ± 0.3	*–2.1 ± 0.4*	*1.4 ± 0.3*
E. frigida juvenile	PF	4	23 ± 5	4.9 ± 0.3	*–1.1 ± 0.1*	*1.1 ± 0.2*	–24.3 ± 0.9	*–1.6 ± 0.6*	*1.4 ± 0.9*
E. superba juvenile	LS	23	64 ± 21	2.1 ± 0.9	*–0.8 ± 0.3*	*0.9 ± 0.2*	–31.1 ± 0.7	*–1.5 ± 0.5*	*1.6 ± 0.4*
E. superba adult	LS	20	228 ± 51	3.6 ± 0.4	*–1.1 ± 0.4*	*1.0 ± 0.4*	–31.3 ± 0.7	*–1.1 ± 1.6*	*1.7 ± 0.7*
E. superba n-gr. female	SI	6	190 ± 47	3.8 ± 0.2	*–1.2 ± 0.2*	*1.0 ± 0.2*	–27.2 ± 0.5	*–0.9 ± 0.7*	*1.0 ± 0.5*
Mean					**–1.0**	**0.9**		**–1.4**	**1.4**
Mature									
E. superba male	SG	3	284 ± 27	5.3 ± 0.4	–0.8 ± 0.5	0.1 ± 0.2	–22.7 ± 2.3	–1.2 ± 1.4	–0.2 ± 0.2
E. superba juvenile	SI	9	188 ± 31	4.2 ± 0.4	*–0.9 ± 0.3*	*0.3 ± 0.1*	–25.1 ± 0.9	*–0.5 ± 0.5*	–0.1 ± 0.4
Mean					**–0.9**	**0.2**		**–0.9**	**–0.1**
E. superba gr. female	SG	3	424 ± 96	4.0 ± 0.3	*–0.9 ± 0.1*	*1.4 ± 0.1*	–22.6 ± 1.0	*–0.7 ± 0.2*	0.5 ± 0.4
E. superba gr. female	SI	9	297 ± 80	2.9 ± 0.4	*–0.9 ± 0.2*	*1.4 ± 0.3*	–28.3 ± 0.7	*–0.6 ± 0.3*	*1.4 ± 0.5*
Mean					**–0.9**	**1.4**		**–0.6**	**1.0**

Fig. 2. *Euphausia superba*. $\delta^{15}N$ (‰ ± 1SD, n = 3), $\delta^{13}C$ (‰ ± 1 SD, n = 3) and C:N ratios (± 1SD, n = 3) of reproductively active males and gravid females, sampled at 3 stations near the South Shetland Islands in March 2000. Values are given for digestive gland region (digest. gland), abdominal segment 3 (abd. segm.) and whole body

Amino acid profile of *Euphausia superba*

In reproductively active males and females from the South Shetland Islands, the total nitrogen content was between 87 (female, digestive gland region) and 110 mg g^{-1}dry $mass^{-1}$ (male, abdominal segment). This nitrogen was largely recovered in amino acids (Table 2). The dominant amino acids were aspartic acid, glutamic acid, glycine and alanine, each accounting for 9 to 15 mol% of the total amino acid

Table 2. *Euphausia superba*. Amino acid composition and $\delta^{15}N$ of individual amino acids within (a) digestive gland region, (b) abdominal segment 3, and (c) remaining body of krill caught near South Shetland Islands in March 2000. Samples were pooled out of 9 individuals, and $\delta^{15}N$ analyses were repeated 2 to 3 times (mean ± SE). Essential amino acids (up to and including valine; Claybrook 1983) are listed first. The sample of males abdominal segment was lost for $\delta^{15}N$ measurement. dw: dry weight

Amino acid	Mol %		$\delta^{15}N$ (‰)		Contribution to $\delta^{15}N$ of amino acid N (‰)	
	Male	Female	Male	Female	Male	Female
a) Digestive gland region						
Argentine[a]	5.4	5.7				
Histidine[a]	0.8	1.6				
Isoleucine[a]	3.1	4.0	12.0 ± 0.7	3.8 ± 0.4	0.4	0.2
Leucine[a]	6.9	7.1	7.9 ± 0.2	7.2 ± 0.4	0.5	0.5
Lysine[a]	7.4	7.4	–1.6 ± 0.0	0.0 ± 0.1	–0.1	0.0
Methionine[a]	0.6	0.4		12.3 ± 0.7		0.0
Phenylalanine[a]	3.2	3.4	3.5 ± 0.2	3.9 ± 0.1	0.1	0.1
Threonine[a]	4.9	5.1	–9.9 ± 0.9	–10.0 ± 0.3	–0.5	–0.5
Valine[a]	4.3	4.8	8.3 ± 0.4	4.9 ± 0.4	0.4	0.2
Alanine	8.8	12.3	7.7 ± 0.4	7.2 ± 0.3	0.7	0.9
Aspartic acid	13.3	12.6	–1.0 ± 1.0	–0.9 ± 0.2	–0.1	–0.1
Glutamic acid	10.6	9.6	15.5 ± 1.4	7.7 ± 0.4	1.6	0.7
Glycine	11.8	8.5	–3.0 ± 0.1	–1.7 ± 0.2	–0.3	–0.1
Proline	5.3	5.2	6.4 ± 0.1	6.7 ± 0.3	0.3	0.3
Serine	5.7	5.8	–1.8 ± 0.2	–2.9 ± 0.3	–0.1	–0.2
Taurine	4.8	3.6				
Tyrosine	3.1	2.8	2.0	3.5 ± 0.6	0.0	0.1
Total amino acids (µmol g^{-1} dw)	2626	3000				
Amino acid N (% of total N)	63	72				
Calculated $\delta^{15}N$ of amino acid N (‰)				2.9	2.1	
Measured bulk $\delta^{15}N$ (‰)					3.3	2.1
b) Abdominal segment 3						
Argentine[a]	7.6	10.0				
Histidine[a]	1.1	1.1				
Isoleucine[a]	2.8	3.1		10.2 ± 0.4		0.3
Leucine[a]	6.3	6.5		12.5 ± 0.4		0.8
Lysine[a]	6.4	6.8		1.1 ± 0.1		0.1
Methionine[a]	0.6	0.8		7.0 ± 0.3		0.1
Phenylalanine[a]	3.0	3.0		–0.7 ± 0.6		0.0
Threonine[a]	4.0	4.2		–5.9 ± 0.3		–0.2
Valine[a]	3.2	3.4		12.3 ± 0.5		0.4
Alanine	8.8	9.2		11.1 ± 0.3		1.0
Aspartic acid	13.2	13.0		4.9 ± 0.2		0.6
Glutamic acid	11.7	11.1		13.8 ± 0.7		1.5
Glycine	15.0	11.8		–6.5 ± 0.4		–0.8
Proline	4.4	6.6		8.5 ± 0.2		0.6
Serine	5.2	5.2		–5.6 ± 0.6		–0.3
Taurine	4.0	1.6				
Tyrosine	2.5	2.7		3.7 ± 0.8		0.1
Total amino acids (µmol g^{-1} dw)	3467	3908				
Amino acid N (% of total N)	69	82				
Calculated $\delta^{15}N$ of amino acid N (‰)						4.2
Measured bulk $\delta^{15}N$ (‰)						4.4
c) Remaining body						
Argentine[a]	6.3	6.4				
Histidine[a]	1.0	1.5				
Isoleucine[a]	2.9	3.5	8.1 ± 0.2	7.9 ± 0.6	0.2	0.3
Leucine[a]	6.3	6.6	12.3 ± 0.7	10.9 ± 0.2	0.8	0.7
Lysine[a]	6.5	7.0	4.9 ± 0.1	3.5 ± 0.6	0.3	0.2
Methionine[a]	0.8	0.4	16.0 ± 0.0	11.5 ± 0.3	0.1	0.0
Phenylalanine[a]	3.6	3.7	–1.4 ± 0.3	–2.5 ± 0.2	–0.1	–0.1
Threonine[a]	4.2	4.8	–4.8 ± 0.6	–8.0 ± 0.0	–0.2	–0.4
Valine[a]	3.5	4.0	9.7 ± 0.3	7.2 ± 0.6	0.3	0.3
Alanine	9.0	10.9	13.6 ± 0.7	9.0 ± 0.8	1.2	1.0
Aspartic acid	12.8	13.0	10.0 ± 0.4	3.7 ± 0.5	1.3	0.5
Glutamic acid	11.1	10.7	13.2 ± 0.5	12.1 ± 0.7	1.5	1.3
Glycine	14.3	10.0	–4.9 ± 0.1	–5.6 ± 0.7	–0.7	–0.6
Proline	5.0	5.8	7.8 ± 0.6	8.3 ± 0.5	0.4	0.5
Serine	5.6	5.8	–4.9 ± 0.4	–5.8 ± 0.7	–0.3	–0.3
Taurine	4.5	3.0				
Tyrosine	2.7	2.7	4.6 ± 0.2	4.3 ± 0.0	0.1	0.1
Total amino acids (µmol g^{-1} dw)	3234	3488				
Amino acid N (% of total N)	73	81				
Calculated $\delta^{15}N$ of amino acid N (‰)					4.9	3.5
Measured bulk $\delta^{15}N$ (‰)					4.2	2.9

pool (Table 2). The proportion of essential amino acids was between 35 and 40%, depending on gender and tissue.

The proportion of most amino acids differed by less than 1 mol % between males and females (Fig. 3). There were, however, some notable variations. Higher proportions of glycine (3 to 4%) and taurine (1 to 2%) were common in all 3 tissues of males compared to females. Conversely, females had 2 to 3% more alanine than males in the digestive gland region and remaining body, and ca. 2% more arginine and proline in the abdominal segment.

Other tissue-specific differences in amino acid composition were independent of gender. Both males and females had a higher proportion of arginine, glycine and glutamic acid in the abdominal segment than in the digestive gland region, while the proportion of taurine, serine and several essential amino acids was higher in the digestive gland region (Fig. 4). The amino acid composition in the remaining body was intermediate between those of digestive gland region and abdominal segment, with relative proportions not differing from either one by more than 1%. Exceptions were arginine in males and females, and alanine, glycine and taurine in females, which differed by 1 to 4 mol% between remaining body and abdominal segment or digestive gland region.

The $\delta^{15}N$ of individual amino acids in *Euphausia superba*

Stable N isotope values varied widely among individual amino acids, spanning a range of more than 25‰ in the digestive gland region of males, 20‰ in the abdominal segment of females and ca. 21‰ in the remaining body of males and females (Table 2). Threonine, glycine and serine had the lowest $d^{15}N$ values in all 3 tissues, while glutamic acid, methionine and leucine were always among those amino acids with highest $\delta^{15}N$.

Differences in amino acid $\delta^{15}N$ between males and females showed a specific pattern in the digestive gland region compared to remaining body (Fig. 3). In both tissues, most amino acids differed by <2‰ between males and females. However, while positive and negative differences were both well represented in the digestive gland region, there was a general tendency of higher values in males relative to females in the remaining body. In addition to these amino acids showing relatively small differences between males and females, several amino acids stood out with particularly high values in males. These were isoleucine, glutamic acid and, to a lesser extent, valine in the digestive gland region, and aspartic acid, alanine, methionine, threonine and valine in the remaining body (Fig. 3).

Most of the amino acids in the digestive gland region of females had lower $\delta^{15}N$ values than their counterparts in the abdominal segment (Fig. 5). The differences were widely distributed from <1 ‰ for tyrosine to >7 ‰ for valine. Exceptions were methionine, glycine, phenylalanine and serine, which all had substantially higher values in the digestive gland region than in the abdominal segment. The $\delta^{15}N$ of amino acids in the remaining body were usually intermediate between the abdominal segment and digestive gland region. Only lysine, phenylalanine and, to a lesser extent, tyrosine showed larger $\delta^{15}N$ differences between digestive gland region and remaining body than between digestive gland region and abdominal segment.

As in females, most of the amino acids in males had lower $\delta^{15}N$ in the digestive gland region than in the remaining body (Fig. 5). Some of the exceptions to this pattern were common to males and females (phenylalanine, serine and glycine), while others were only seen in males (isoleucine and glutamic acids). The magnitudes of differences (both positive and negative) varied from 1.4‰ for proline and valine up to 11‰ for aspartic acid.

Comparison between bulk $\delta^{15}N$ and calculated $\delta^{15}N$ of amino acid nitrogen

The measured bulk $\delta^{15}N$ values were compared to calculated $\delta^{15}N$ of amino acid nitrogen. Individual amino acids contributed to these $\delta^{15}N$ of amino acid nitrogen by the product of their molar proportion and $\delta^{15}N$ (Table 2). The calculated values were based on ca. 60% of the total nitrogen as cysteine, tryptophan, arginine, histidine, taurine and any non-amino acid nitrogen are not included. Nevertheless, the calculated $\delta^{15}N$ of amino acid nitrogen reflected differences between gender and tissues in a very similar manner to the bulk $\delta^{15}N$ values (Table 2). In the remaining body of males and females, for instance, calculated values were ca. 0.7‰ higher than bulk $\delta^{15}N$ values, but the difference between gender was consistent between the 2 approaches, 1.3 to 1.4‰. Likewise, females had lower calculated $\delta^{15}N$ of amino acid nitrogen and bulk $\delta^{15}N$ in the digestive gland region than males, even though the difference was slightly more pronounced in bulk $\delta^{15}N$ values. Finally, tissue-specific differences in the bulk $\delta^{15}N$ were also shown in calculated $\delta^{15}N$ of amino acid nitrogen: males and females had higher values in the remaining body than in the digestive gland region, and females had their highest values in the abdominal segment.

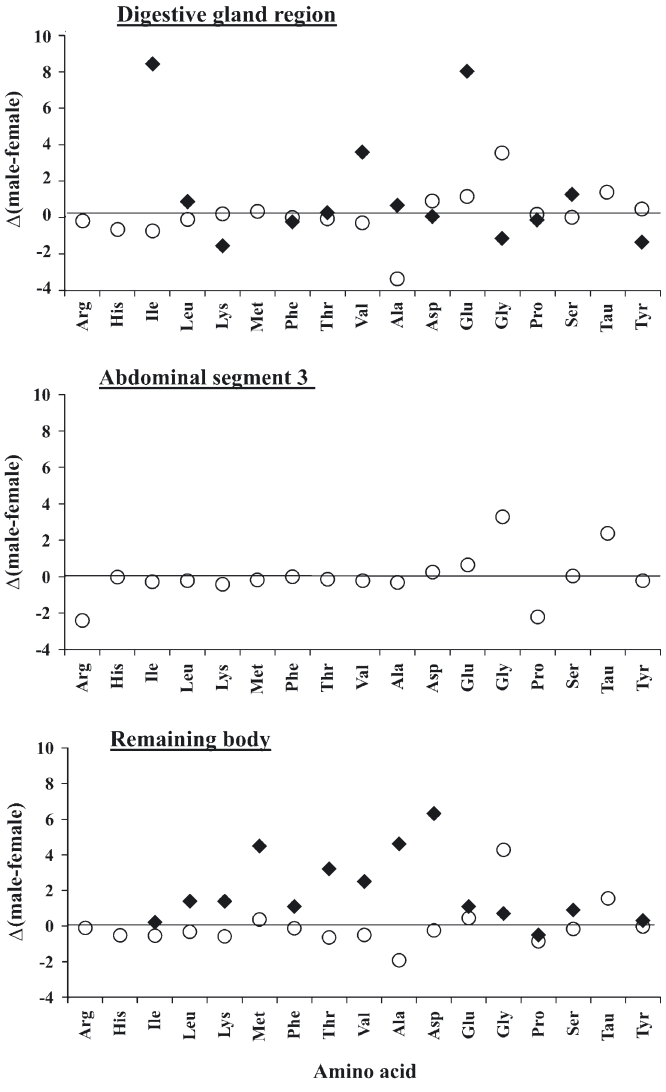

Fig. 3. *Euphausia superba.* Gender-specific differences in concentration (mol%; ○) and $\delta^{15}N$ (‰; ◆) of individual amino acids. Arg, arginine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Phe, phenylalanine; Thr, threonine; Val, valine; Ala, alanine; Asp, aspartic acid; Glu, glutamic acid; Gly, glycine; Pro, proline; Ser, serine; Tau, taurine, Tyr, tyrosine

Contribution of individual amino acids to the $\delta^{15}N$ of amino acid nitrogen

In the remaining body, the difference between males and females was mainly caused by aspartic acid (Table 3). The contribution of aspartic acid to the $\delta^{15}N$ of amino acid nitrogen differed by 0.8‰ between genders, while for all other amino acids the difference did not exceed 0.2‰. The strong effect of aspartic acid was based on its high proportion in both males and females, and its markedly lower $\delta^{15}N$ in females. Other amino acids, which also led to a lower $\delta^{15}N$ of amino acid nitrogen in females than in males, were alanine, threonine and glutamic acid. Their varying contributions were mainly caused by differences in $\delta^{15}N$, which were either slightly increased (threonine, glutamic acid) or reduced (alanine) by differences in their relative proportion. In contrast, the contribution of glycine, proline and isoleucine was ca. 0.1 ‰ lower in males than in females. Even though the $\delta^{15}N$ of glycine was higher in males, a large difference in its relative proportion between males and females negated this isotopic difference.

In the digestive gland region, glutamic acid caused a 0.9‰ difference between gender (Table 3). The relative proportion of glutamic acid was high in both males and females, but the $\delta^{15}N$ was markedly lower in females. Other important amino acids were isoleucine and valine, each accounting for a ~0.2‰ difference. Their lower contribution in females was mainly caused by lower $\delta^{15}N$ values, while this effect was slightly

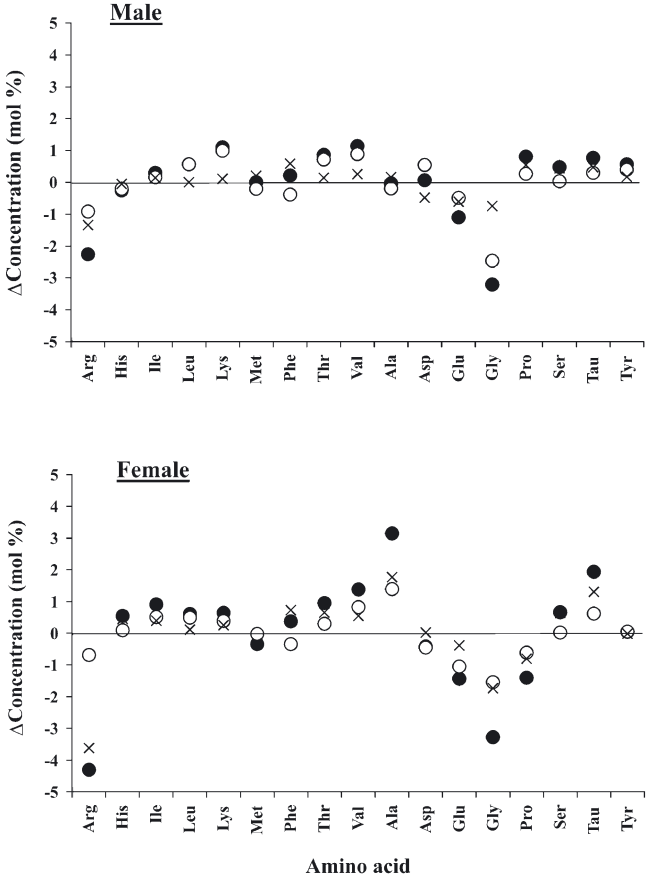

Fig. 4. *Euphausia superba.* Tissue-specific differences in concentration of amino acids between: ●, digestive gland region and abdominal segment; ○, digestive gland region and remaining body; and ×, abdominal segment and remaining body. See Fig. 3 legend for amino acid abbreviations

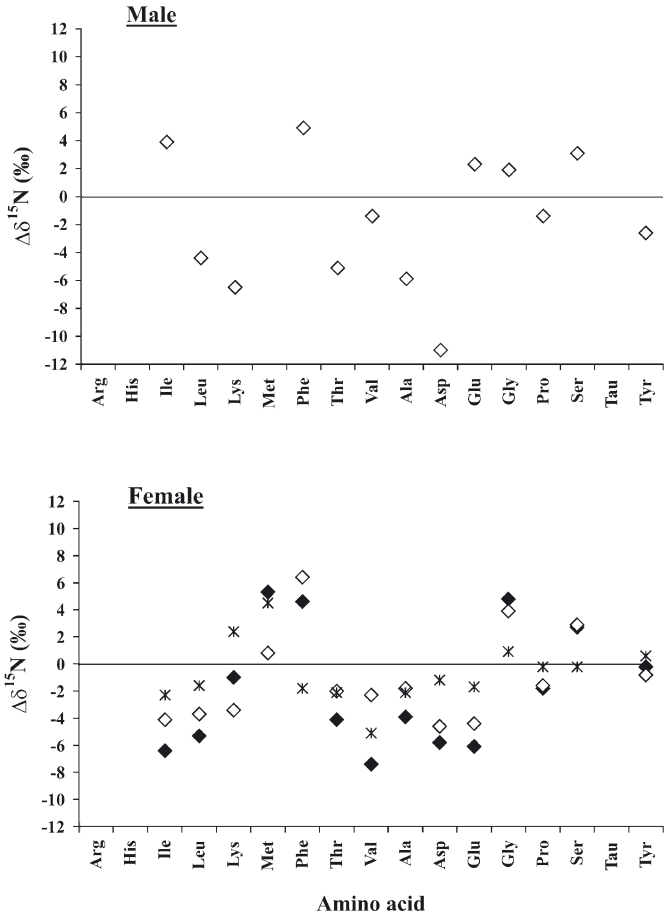

Fig. 5. *Euphausia superba*. Tissue-specific differences in $\delta^{15}N$ of individual amino acids between: ◆, digestive gland region and abdominal segment; ◇, digestive gland region and remaining body; and ✳, abdominal segment and remaining body. The sample of males abdominal segment was lost for $\delta^{15}N$ measurement. See Fig. 3 legend for amino acid abbreviations

reduced by differences in relative proportion. Again, some of the amino acids contributed lower values in males than in females. These were mainly glycine, alanine and lysine (each 0.1 to 0.2 ‰ difference). For glycine and lysine, males had lower $d^{15}N$ values and their relative proportions amplified this effect. In contrast, alanine had a slightly higher $d^{15}N$ in males, but a clearly higher proportion in females.

Tissue-specific differences in $\delta^{15}N$ of amino acid nitrogen between digestive gland region and remaining body or abdominal segment were caused mainly by some of the most abundant amino acids: glutamic acid and aspartic acid in females, aspartic acid and alanine in males (Table 3). Those amino acids contributed to the $\delta^{15}N$ of amino acid nitrogen with a 0.5 to 1.4‰ lower value in the digestive gland region than in the remaining body or abdominal segment. All 4 amino acids had a markedly lower $\delta^{15}N$ in the digestive gland region, while their relative proportions were similar between tissues. Glycine showed the opposite trend, contributing 0.4 to 0.7‰ higher values to the digestive gland region than to the remaining body of females and males or to the abdominal segment of females. This was caused both by a lower $\delta^{15}N$ and a higher relative proportion of this amino acid in the remaining body and abdominal segment compared to the digestive gland region. In females, the isotopic difference between abdominal segment and remaining body resulted from several amino acids contributing slightly higher values (0.1 to 0.2 ‰) to the abdominal segment, while only glycine and lysine had some contrary effect.

DISCUSSION

Micronekton, including *Euphausia superba*, have usually been analysed as whole animals for their isotopic composition (Wada et al. 1987, Rau et al. 1991, Hodum & Hobson 2000). However, large consumers are known to vary in $\delta^{15}N$ of individual body components, even when equilibrated with the diet (DeNiro & Epstein 1981, Hobson & Clark 1992a). In this study, euphausiids showed consistently 1 to 2‰ differences in $\delta^{15}N$ between tissues, and between reproductively active males and females. The tissue- and gender-specific variations in $\delta^{15}N$ were examined in relation to compositional and isotopic differences in the main body nitrogen component, the amino acids. From this, we suggest that $\delta^{15}N$ differences between mature males and females were caused by their specific compositions and physiology rather than by feeding at different trophic levels. Enlarged ovaries swelled the thoraxes of the females, and accounted for 30 to 50% of their weight (Mayzaud et al. 1998, Cuzin-Roudy 2000). In males, the main body fraction is the abdomen, while gonads represent only ca. 14% of the weight (Mayzaud et al. 1998).

The results show that both the composition and the $\delta^{15}N$ of individual amino acids varied between tissues and gender, and together these explained most of the observed differences in bulk $\delta^{15}N$. However, compositional and isotopic effects interplay in a complex manner, as each amino acid contributes to the bulk $\delta^{15}N$ by the product of its relative proportion and its $\delta^{15}N$ value. Thus, differences in the proportion of an individual amino acid among sample types can be amplified or reduced by their differences in $\delta^{15}N$. Taking this relationship into consideration, it is apparent that although there were measurable differences in many of the amino acids (mol% and/or $\delta^{15}N$) between gender or tissues, the observed differences in bulk $\delta^{15}N$ were predominantly caused by only a few of the amino acids.

Table 3. *Euphausia superba*. Difference in the contribution of individual amino acids to the $\delta^{15}N$ of amino acid N (‰) in various tissues of males and females. Essential amino acids (up to and including valine; Claybrook 1983) are listed first. Digest.: digestive gland region; remain.: remaining body; abd.: abdominal segment 3

Amino acid	Δ (male-female)		Δ (remain.-digest.)		Δ (abd.-digest.)	Δ (abd.-remain.)
	Digest.	Remain.	Male	Female	Female	Female
Isoleucine[a]	0.2	−0.1	−0.2	0.1	0.1	0
Leucine[a]	0	0.1	0.3	0.2	0.3	0.1
Lysine[a]	−0.1	0.1	0.4	0.2	0.1	−0.1
Methionine[a]		0.1	0.1	0	0.1	0.1
Phenylalanine[a]	0	0	−0.2	−0.2	−0.1	0.1
Threonine[a]	0	0.2	0.3	0.1	0.3	0.2
Valine[a]	0.2	0	−0.1	0.1	0.2	0.1
Alanine	−0.2	0.2	0.5	0.1	0.1	0
Aspartic acid	0	0.8	1.4	0.6	0.7	0.1
Glutamic acid	0.9	0.2	−0.1	0.6	0.8	0.2
Glycine	−0.2	−0.1	−0.4	0.5	−0.7	−0.2
Proline	0	−0.1	0.1	0.2	0.3	0.1
Serine	0.1	0	−0.2	−0.1	−0.1	0
Tyrosine	−0.1	0	0.1	0	0	0
∑ negative	0.6	0.3	1.2	0.8	0.9	0.3
∑ positive	1.4	1.7	3.2	2.2	3.0	1

[a]Essential amino acid

These were aspartic acid and glutamic acid, which showed both consistently high abundances and pronounced variations in $\delta^{15}N$ between gender or tissues. Another important amino acid, glycine, was characterised by variable, but high, proportions as well as differences in $\delta^{15}N$.

The tissue- and gender-specific variations in amino acid composition and isotopic value raise questions over the physiological processes involved. However, a detailed explanation of individual results is beyond the scope of the present study as the mechanisms are likely complex and largely unknown for krill. Nevertheless, we have compiled information from the literature to make some suggestions, firstly about physiological differences between male and female krill, and secondly, about differences between tissues. These suggestions illustrate that compositional and isotopic differences between gender and tissues might not be restricted to krill but are of general character to consumers.

Male-female differences in amino acid composition

Krill males and females are known to vary in their lipid content and lipid class composition (Pond et al. 1995, Virtue et al. 1996). However, differences in their amino acid composition have not been studied for *Euphausia superba* to our knowledge. Data from postlarvae (stage and gender unspecified) are variable, probably depending on sample preparation and analysis, as well as on the life history of the krill (e.g. Srinivasagam et al. 1971, Ferguson & Raymont 1974, Lee et al. 1979, Partmann 1981, Zhang et al. 2002). However, regardless of the exact order, glutamic acid, aspartic acid, glycine, alanine, lysine and leucine were the major amino acids on a molar basis in all studies, including ours.

Reproductively active krill males and females differed clearly in their amino acid composition. Males had higher proportions of glycine and taurine, while females were richer in alanine (in the digestive gland region and remaining body) or arginine and proline (in the abdominal segment). We suggest that these differences are due to the high proportion of yolk protein in females. Differences in the amino acid composition of eggs vs. whole body tissue, or oocytes vs. muscle, have been described for fish and shrimp (Wilson & Poe 1985, Dy-Peñaflorida & Millamena 1990, Ng & Hung 1994, Gunasekera 1997). Some of the variations found in krill were common to these other species: e.g. eggs or oocytes had low proportions of glycine and high proportions of alanine compared to the whole body or muscle. High proportions of alanine have also been reported for vitellin, the major yolk protein in crustaceans (Tom et al. 1992, Chang et al. 1993, Qui et al. 1997, Volz et al. 2002). Thus, the gonad material could explain male-female differences in the digestive gland region and remaining body. However, it cannot explain differences seen in the third abdominal segment, as the ovaries do not reach this segment, even at their full extension (Cuzin-Roudy 2000).

In males, a lower proportion of proline and arginine in the third abdominal segment might indicate an

energy deficit relative to females. Proline and arginine are abundant free amino acids in krill and other crustaceans (Partmann 1981, Claybrook 1983). An oxidation of proline for energy production is well known in invertebrates (Adams & Frank 1980, Hochachka et al. 1983), and it virtually disappears from the free amino acid pool during starvation (Torres 1973, Dall & Smith 1987). In muscle, arginine could either be converted to phosphoarginine, which provides a phosphagen for energy transformation, or via ornithine to proline, which is then oxidized (Hird et al. 1986).

However, free amino acids also have a role in osmotic regulation (McCoid et al. 1984), so the total free amino acid concentration would need to remain nearly constant during starvation. In a study on fasting tiger prawn *Penaeus esculentus*, the loss of proline was almost completely compensated by an increase in glycine (Dall & Smith 1987). This could explain the higher relative proportion of glycine in males' abdominal segments compared to females. Taurine, another abundant free amino acid and osmotic effector (Claybrook 1983), was also more abundant in krill males than females.

Male-female differences in $\delta^{15}N$ values

We suggest that the variations in $\delta^{15}N$ between immature krill, mature males and females were caused by differences in protein metabolism, in addition to a varying body composition. Primary sources of nitrogen isotopic fractionation are amino acid deamination and transamination (Macko et al. 1986). Excreted nitrogen contained in ammonia and urea is isotopically lighter than body protein (Steele & Daniel 1978). Thus, higher $\delta^{15}N$ values in males than in immature krill might indicate a higher ratio between protein degradation and protein synthesis, and therefore relatively more excreted nitrogen. In contrast, lower $\delta^{15}N$ values in the whole body of females, and larger $\delta^{15}N$ differences between whole body and abdominal segment, suggests a lower ratio of protein degradation to protein synthesis, and relatively less nitrogen excretion.

Reproductively active krill males are known to have low lipid levels with negligible triacylglycerol stores, but high mortality (Pond et al. 1995, Virtue et al. 1996). This depletion of lipid reserves is attributed to the energetic costs of reproduction (spermatophore production and sustained physical activity during searching for females and mating), rather than to food limitation (Pond et al. 1995, Virtue et al. 1996). After using up lipids, protein becomes an increasingly important energy source in krill (Ikeda & Dixon 1982, Quetin & Ross 1991, Nicol et al. 1992). As mentioned above, proline and arginine, 2 amino acids preferentially used for energy production, had lower relative proportions in male than in female krill.

During a reproductive season, krill females can have 3 to 9 spawning episodes, with more than 1000 eggs being released each time (Ross & Quetin 2000). This involves an intensive production of new tissues (growth), and high rates of protein- and lipid-syntheses. However, higher protein synthesis rates do not necessarily result in a similar increase in energetic expenses, as costs associated with activation of tRNA and synthesis of rRNA are fixed (Houlihan et al. 1995). In addition, organisms with a high growth potential channel assimilated energy into growth rather than utilize it for increased turnover of body constituents (Kiørboe et al. 1987). Thus, increased growth rates are often accompanied by reduced levels of protein turnover and degradation (Hawkins 1985, Conceição 1997). As a consequence, krill oocytes would have lower $d^{15}N$ values than the females. This has been described for 5 decapod species, where recently ecloded zoeae larvae were ^{15}N-depleted by up to 2.3‰ in relation to their parents (Schwamborn et al. 2002).

This study is the first, to our knowledge, that has compared $\delta^{15}N$ values of individual amino acids between tissues or gender of aquatic animals. However, McClelland & Montoya (2002) studied rotifers cultured in the lab and size-fractionated zooplankton from the field and showed that some amino acids become strongly fractionated in food-web relationships (e.g. alanine, glutamic- and aspartic acid), while others are transferred rather conservatively (e.g. glycine, phenylalanine, serine and tyrosine). Notably, those amino acids identified by McClelland & Montoya (2002) as most fractionated in the food web also showed large differences between male and female krill in either the digestive gland region or remaining body. Conversely, amino acids that varied little between trophic levels were also consistent in krill males and females. These similarities might be caused by the specific metabolic function, pathways of synthesis and degradation, as well as turnover rates of individual amino acids.

Male-female differences in trophic level

Males had ca. 1.3‰ higher $\delta^{15}N$ values in the whole body than females, which would usually have been interpreted as a higher trophic position (Peterson & Fry 1987) or long-term starvation (Hobson et al. 1993). However, several points suggest that this need not be the case. Firstly, fatty acid profiles and the presence of algal sterols in the digestive glands indicated that *Euphausia superba* males feed during the reproduction period (Virtue et al. 1996). In addition, Priddle (et al. 1990) found

that gut fullness and feeding capacity of mature males did not differ from those of gravid females. Secondly, the $\delta^{15}N$ values were similar in the abdominal segments of males and females, but not in the digestive gland region and whole body, at both sampling locations. This suggests that male-female differences in $\delta^{15}N$ of the whole body were related to tissue-specific composition and function rather than to feeding.

Thirdly, the amino acid $\delta^{15}N$ relationships described by McClelland & Montoya (2002) for smaller zooplankton species can be used as a benchmark for interpreting the trophic level of krill. The isotopic difference between a strongly fractionated amino acid (glutamic acid) and one which is not fractionated within the food web (phenylalanine) was proposed as an internal index to trophic position: $D\delta^{15}N_{glu\text{-}phe} = \delta^{15}N_{glu} - \delta^{15}N_{phe}$. This index is ca. 4‰ in phytoplankton, 11‰ in herbivorous zooplankton, and 18‰ in carnivorous zooplankton, with a shift of one trophic level resulting in an increase of 7‰ (McClelland & Montoya 2002).

Applied to krill, $D\delta^{15}N_{glu\text{-}phe}$ was 14.6‰ in the remaining body of both males and females, suggesting omnivorous feeding. A value of 14.5‰ was also found the abdominal segment of females. In the digestive gland region, the factor was 12‰ in males, but unrealistically low in females (3.8‰). The latter might indicate specific amino acid metabolism in the female digestive gland region. However, as the remaining body and abdominal segment account for more than 90% of the total nitrogen, $D\delta^{15}N_{glu\text{-}phe}$ of the whole body of females was only slightly lower than in males (13.8‰ compared to ca. 14.4‰). In addition, the very similar $\delta^{15}N$ of phenylalanine, serine and tyrosine in males and females suggest that they were feeding within the same food web (McClelland & Montoya 2002).

Tissue-specific differences in amino acid composition and $\delta^{15}N$ values

The consistency of tissue-specific differences in $\delta^{15}N$, seen for several euphausiid species and stages sampled at various stations and seasons, suggested that it is due to distinct tissue composition and physiology rather than to a recent change in food sources and the differing integration periods of these tissues.

Both male and female krill had higher proportions of glycine and arginine in the abdominal segment than in the digestive gland region or remaining body, while differences in most other amino acids were within 1 mol%. This supports previous findings of high abundances of arginine and glycine in crustacean muscle (Claybrook 1983, Dy-Peñaflorida & Millamena 1990).

In females, 10 out of 14 amino acids had higher $\delta^{15}N$ values in the abdominal segment or remaining body than in the digestive gland region; the corresponding number for males was 9. With our present knowledge, these tissue-specific differences in amino acid $\delta^{15}N$ cannot be easily attributed to particular processes. The rate of protein synthesis and degradation, internal export of metabolites, the fate of amino acids released from degraded proteins, amino acid pool size, and urea recycling can all have an influence on the $\delta^{15}N$ values, and some combination of these factors is no doubt involved. Nonetheless, some basic differences between digestive gland and abdominal segment will be pointed out, and a few processes, which might lead to specific fractionation of amino acid nitrogen, will be discussed.

The digestive gland of crustaceans is responsible for the synthesis and secretion of digestive enzymes and the subsequent uptake of nutrients (Claybrook 1983). In metabolically active tissues, protein synthesis and turnover is usually very high, but only a relatively small proportion of synthesis is retained as growth. This has been shown for the digestive gland of crustaceans (Houlihan et al. 1990, Hewitt 1992), and also for the liver of fish (Carter & Houlihan 2001). In contrast, the abdominal segment is mainly built of muscles. Protein synthesis in muscles involves, first of all, the synthesis of myofibrillar protein, with rates being lower than in the digestive gland or liver, but up to 50% and more is retained as growth (Hewitt 1992, Carter & Houlihan 2001). As nitrogen isotopes become mainly fractionated during amino acid deamination and transamination (Macko et al. 1986), enrichment of ^{15}N is more likely in tissues with high protein turnover and degradation than in growing tissue. Accordingly, bulk $\delta^{15}N$ values are expected to be lower in the muscle than in the digestive gland or liver. This has indeed been found for mammals (DeNiro & Epstein 1981, Hobson et al. 1996) and for birds (Hobson & Clark 1992a, Hobson et al. 1993), but not for fish (liver had lower $\delta^{15}N$ than muscle, Pinnegar & Polunin 1999) or krill (present study).

However, the above-mentioned tissue-specific balances between protein synthesis/degradation and growth are only valid when there is no shortage of food. During starvation, muscle protein synthesis rates decrease, proteins become hydrolysed, and free amino acids are catabolised to provide energy (Dall & Smith 1987, Carter & Houlihan 2001). For krill, which feeds and migrates in swarms, short-term reduction in food supply is a common feature year-round. During such times, muscle proteins are likely to be involved in energy supply within the abdomen, while lipids become catabolised in the digestive gland (Virtue et al. 1993, Nicol 2000). Thus, higher $\delta^{15}N$ in

krill muscle might, at least partly, be related to increased rates of amino acid catabolism within periods of food shortage.

Glutamine catabolism is an additional process, which might cause high $\delta^{15}N$ in muscle of fish (and krill) but not in higher vertebrates. In the muscle, catabolised amino acids release ammonium, which is collected as glutamate. This glutamate is not catabolised to a significant extent in mammalian muscle, but becomes transported to the liver either as glutamine or as alanine (Nelson & Cox 2001). Rather than exporting glutamine to other tissues, fish muscle has a high capacity for the catabolism of glutamine (Ballantyne 2001). Thus, within fish muscle, glutamine has a central role as an oxidative substrate and in ammonia excretion (Ballantyne 2001). In female krill, glutamic acid had a ~6 ‰ higher $\delta^{15}N$ in the abdominal segment than in the digestive gland. Thus, high abundance and isotopic variation in glutamic acid accounted for most of the differences in bulk $\delta^{15}N$ between the 2 tissues.

Almost all of the individual amino acids supported the tissue-specific differences in bulk $\delta^{15}N$, with only a few showing the opposite trend. However, looking in detail, individual amino acids clearly varied in the extent of their tissue-specific differences in $\delta^{15}N$. Relating those tissue-specific differences in $\delta^{15}N$ to variations in amino acid concentration, it becomes clear that for essential amino acids, some of the variability in $\delta^{15}N$ can be explained by the actual amino acid pool size (50 to 70%, Fig. 6). Thus, it is more likely that higher $\delta^{15}N$ values of an individual amino acid are found in a tissue with a smaller pool-size. For an essential amino acid, the food source determines the initial isotope value, which is modified within different tissues only according to the amount this amino acids becomes catabolized (deaminated). In contrast, non-essential amino acids are either delivered by the food or re-built from other amino acids (transaminated) and again catabolized (deaminated). Therefore, effects on their $\delta^{15}N$ are more complex and a relationship between $\delta^{15}N$ and amino acid pool size was not obvious.

CONCLUSION

This study is one of the few that has looked in detail at the building blocks of the bulk $\delta^{15}N$ in consumers. For euphausiids, we found that the proportion and $\delta^{15}N$ of individual amino acids differed markedly between body parts containing mainly muscle, digestive gland or oocytes. This explained most of the 1 to 2‰ differences in bulk $\delta^{15}N$ between krill gender or tissues. We therefore suggest that bulk $\delta^{15}N$ values are affected by the specific body composition, growth potential and metabolism. This has wider implications for the isotopic analyses of metazoans in general. The commonly used approach to interpret trophic level from whole body samples of micronekton can cause bias due to their differing proportions of body tissues and physiology. Restricting the analysis to a more comparable tissue such as muscle would reduce this problem.

Euphausia superba lives in an environment with very variable food abundance. In the past, it was mainly their lipid composition and storage which was studied in relation to extended periods of food shortage, while little attention being paid to their protein metabolism. In this study, variable proportions and $\delta^{15}N$ values of certain amino acids suggested that they are involved in energy supply. Further laboratory experiments are needed to verify our hypotheses. However, the isotopic analysis of amino acids could help us reveal the metabolic pathways in krill.

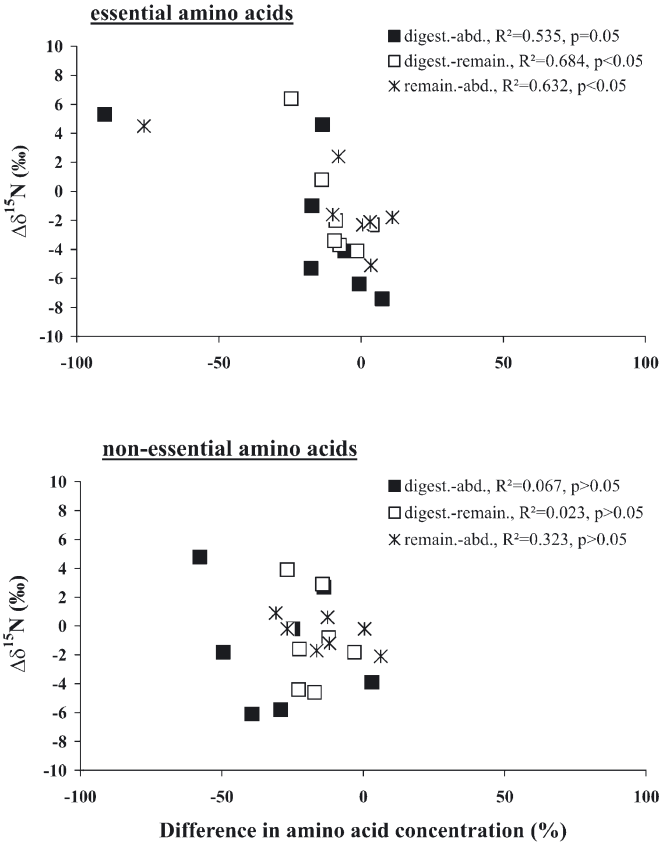

Fig. 6. *Euphausia superba.* Tissue-specific differences in $\delta^{15}N$ of individual amino acids in relation to variations in amino-acid pool size (percentage difference in amino acid concentration relative to the average concentration of that amino acid) between digestive gland region and abdominal segment (digest.-abd.); digestive gland region and remaining body (digest.-remain); and remaining body and abdominal segment (remain.-abd.)

Acknowledgements. G. Cripps and D. Stübing kindly provided krill samples from their cruises in 1996 and 2000, respectively. For their assistance during fieldwork, we thank the officers and crew of the RRS 'James Clark Ross', the RV 'Polarstern' and the RV 'Yuzhmorgeologiya'. We acknowledge the help of I. Liskow, C. Holl and I. Davidson with sample analyses. R. Bastrop, J. Cuzin-Roudy and K. Fraser provided constructive discussion of the results. Valuable comments from 4 anonymous referees are much appreciated. The study was funded by the German Federal Ministry of Education and Research as part of the joint project 'Seasonal feeding strategies of *Euphausia superba* — Antarctic krill' (03PL025C).

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Editorial responsibility: Otto Kinne (Editor), Oldendorf/Luhe

Submitted: August 4, 2003; Accepted: December 2, 2003
Proofs received from author(s): January 26, 2004