197-206

## ANTARCTIC FISHES: THE CHEMICAL COMPOSITION OF MUSCLE, LIVER AND FOOD OF TWO NOTOTHENIOIDS

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ABSTRACT. A combination of low fat (6.1 and 4.8% of dry matter), high protein (78.6 and 84.3% d.m.) and mineral content (6.3 and 6.2% d.m.), as well as high moisture (80.8 and 81.7% of fresh matter), which is typical of Antarctic benthic fish, was found in the muscle of *Notothenia coriiceps* and *Lepidonotothen nudifrons*, respectively. Fish food items (whole amphipods) were high in minerals (34.3% d.m.) and poor in protein (39.1% d.m.), while macroalgae presented an association of high moisture (89% of wet matter), high minerals (36.5% d.m.), low lipids (1.8% d.m.) and very low protein (17.5% d.m.). The poor nutritional value of these food items seems to be compensated for by high food intake. Sulfur levels in fish tissues were negatively related to fish size. Sulfur and nitrogen were inter-correlated strongly and positively, and most of the sulfate is probably derived in muscle and liver from sulfur-containing amino acids.

Key words: NOTOTHENIA CORIICEPS, LEPIDONOTOTHEN NUDIFRONS, CHEMICAL COMPOSITION, MUSCLE, LIVER, FOOD CHAIN

## INTRODUCTION

Antarctic fish species grow slowly, mature late and produce few large eggs that have a protracted incubation time. These factors all contribute to the generally high vulnerability of these species to over-fishing. Commercial exploitation of Antarctic fishes began in the 1969-1970 season. Over recent decades, a dramatic shift in dominance among two closely related species has been observed in the region of the South Shetlands, namely from *Notothenia rossii* Richardson, 1844 (syn. *N. marmorata*), a key commercial species, to *N. coriiceps* Richardson, 1844 (syn. *N. neglecta*). Their preference for shallow waters and the lack of spawning migrations may keep some *N. coriiceps* out of the range of fishing vessels (Kamler 2002). The small species *Lepidonotothen* (*Lindbergichthys*) *nudifrons* (Lönnberg 1905) has yet to be exploited.

Modes of life are reflected in the chemical composition of the tissues of Antarctic fish species; muscle lipids in pelagic species constitute up to 58% of the muscle dry matter, while benthic species are lean (reviews in Friedrich and Hagen 1994, Kamler et al. 2001). *Notothenia coriiceps* and *L. nudifrons* are important demersal notothenioids in Admiralty

Bay (King George Island, South Shetlands) (Skóra 1993, review in Kulesz 1998) and are abundant in the inshore waters of the southern Scotia Arc and the west Antarctic Peninsula (Barrera-Oro 2002). A combination of low fat, high protein and mineral content in dry matter, and high hydration of fresh matter (Love 1970, Kołakowska and Kołakowski 2000), which is typical of benthic fishes, can be expected in *N. coriiceps* and *L. nudifrons*.

Amphipoda were the main prey of both *N. coriiceps* and *L. nudifrons* in Admiralty Bay, macroalgae (Rhodophyta and Phaeophyta) were also regularly consumed by *N. coriiceps*, and single specimens of *L. nudifrons* were found in the stomachs of a few of the largest *N. coriiceps* (Kamler et al. 2001). The trophic ecology of the fish communities of the southern Scotia Arc and the west Antarctic Peninsula was recently reviewed by Barrera-Oro (2002). He demonstrated that demersal fish occupy a key position in the food web of inshore waters.

The main purpose of this study was to analyze the four major elements (nitrogen, carbon, hydrogen and sulfur) and perform proximate analysis (water, ash, protein and lipids) of *N. coriiceps* and *L. nudifrons* tissues. The impact of fish attributes on the chemical composition of tissue is considered, and the concentration of the constituents in fish tissues is compared with their concentration in fish food.

## MATERIAL AND METHODS

Fish (late juveniles and adults) were collected from Admiralty Bay (King George Island, South Shetlands, Antarctica, 62° 10′S, 58°29′W) during the 23<sup>rd</sup> Polish Antarctic Expedition in January-February 1999. *Notothenia coriiceps* and *L. nudifrons* were sampled with baited traps deployed at depths of about 15 and 40 m. Species were identified according to Fischer and Hureau (1985) and deWitt et al. (1990), and scales were collected from just below the upper right lateral line. Samples of white muscle were taken from the middle of the left lateral region of the body. The liver was sampled as a whole. Three samples of fish food (a mixed sample of amphipods and two samples of macroalgae) were taken from a depth of 15 m. Dry samples of fish muscle, liver and food were placed in airtight vials and stored at 2-4°C for one month.

A summary of the fish attributes assessed and the material properties determined is shown in Table 1. Dry weights were taken after drying to a constant weight at 60°C in a desiccator over silica gel. Ash was determined in sub-samples of about 20 mg which were ashed in platinum cups at 550°C for 24 h. Elemental analyses were performed using a Carlo Erba (1108) Elemental CHNS-O Automatic Analyzer with sulfanilamide as a reference. In an earlier study (Kamler et al. 1994), the caloric values computed with the equation shown in Table 1 corresponded well with those measured directly in a bomb calorimeter.

Summary of materials and methods

### TABLE 1

	5				
Fish* attributes					
Attribute	Method				
Sex	Determined by gonad examination				
Age (A, years)	Assessed from scales				
Standard length (Ls, cm)	Measured to the nearest 0.1 cm				
Total body wet weight (W <sub>w</sub> , g)	Taken to the nearest 1g (Notothenia) or 0.01 g (Lepidonotethon)				
Liver wet weight (W1, g)	Taken to the nearest 0.01g				
Condition factor (CF)	Computed as: $CF = 100W_w L_s^{-3}$				
Hepatosomatic index (HSI)	Computed as: $HSI = 100W_1W_w^{-1}$				
	Material properties				
Property	Method				
Dry matter (DM, % wet wt)	Dry and wet weight taken to the nearest 0.001g				
Ash (ASH, % dry wt)	Weight taken to the nearest 0.01 mg				
Carbon (C, % dry wt)	Determined directly (CHNS-O analyzer)				
Hydrogen (H, % dry wt)	Determined directly (CHNS-O analyzer)				
Nitrogen (N, % dry wt)	Determined directly (CHNS-O analyzer)				
Sulphur (S, % dry wt)	Determined directly (CHNS-O analyzer)				
Oxygen (O, % dry wt)	Computed as: $O = 100-(C+H+N+S+ASH)$				
Caloric value (CV, J mg <sup>-1</sup> dry wt)	Computed as: $CV = 0.004184(82C+292.69H-0.25O+25S)^{\$}$				
Protein (P, % dry wt)	Computed as: P = 5.78N**				
Total lipids (L, % dry wt)	Determined directly (phospho-vanillin method)				

\*Fishes studied: N. coriiceps - 19 specimens and L. nudifrons - 10 specimens.

Materials studied: fish muscle and liver, and fish food. Fish food samples were: a mixed sample of Amphipoda (Cheirimedon femoratus (Pfeffer 1888) – 52.6% of wet weight, Orchomenella rotundifrons (K.H. Barnard 1932) – 38.6%, Gondogeneia antarctica (Chevreux 1906) – 7.8% and Hippomedon kerguleni (Miers 1875) – 1%), a sample of Rhodophyta (Curdiea racovitzae Hariot in De Wilderman, 1900) and a sample of Phaeophyta (Himantothallus grandifolius (A. et E.S. Gepp) Zinova 1959). Determined in triplicate.

<sup>§</sup>Dulong's equation, further developed by Colombo et al. (1988) and modified for the use with the 1108 Analyzer.

\*\*Nitrogen-protein conversion factor 5.78, appropriate for aquatic organisms (Gnaiger and Bitterlich 1984) was used; all the nitrogen was assumed to be protein-bound.

Lipid extraction as shown in Kamler et al. (2001), lipid determination according to Knight et al. (1972) and Chromy et al. (1975).

## RESULTS

### PRECISION OF THE MEASUREMENTS

The reproducibility of triplicate measurements of fish tissue samples was evaluated with coefficients of variation (SD  $\times$  100/mean). They ranged from 0.04 to 2.41%, 0.01 to 3.01%, 0.04 to 4.86 % and 0.17 to 13.82% for carbon, nitrogen, hydrogen and sulfur, respec-

tively, and from 1.53 to 16.6% for ash in all the 58 tissue samples analyzed. Thus, the precision of the measurements was good. It was the best in carbon and the poorest in ash.

### **COMPARISONS BETWEEN SPECIES**

The attributes of the fish are compared in Table 2. The two species were of similar age. *N. coriiceps* was much larger and more robust (with a higher CF) than *L. nudifrons,* but it had a lower hepatosomatic index.

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			TABLE 2				
Comparison of attributes between N. coriiceps and L. nudifrons							
Attribute	<i>N. coriiceps</i> (n = 7F + 8M*)	L. nudifrons (n = 9F + 1M)	t-test				
	Mean $\pm$ SD	$Mean \pm SD$	Р				
Age (A, years)	$5.8 \pm 1.7$	$4.9\pm2.1$	> 0.05				
Standard length (L <sub>s</sub> , cm)	33.0 ± 5.4 >	$14.1\pm1.3$	0.000				
Total body wet weight (W <sub>w</sub> , g)	918.7 ± 428.9 >	$49.1 \pm 15.1$	0.000				
Liver wet weight (W <sub>1</sub> , g)	26.3 ± 18.7 >	$1.9\pm0.8$	0.000				
Condition factor (CF)	2.4 ± 0.3 >	$1.7\pm0.1$	0.000				
Hepatosomatic index (HSI)	2.6 ± 1.0 <	$3.8 \pm 1.7$	0.002				

\* F - females, M - males, sex was not identified in remaining four individuals;

> higher mean value (P < 0.05) in N. coriiceps; < lower mean value (P < 0.05) in N. coriiceps;

 $\approx$  difference non-significant (P > 0.05).

In Table 3 the chemical properties of fish muscle and liver are compared with those of the food items, macroalgae and whole amphipods with chitin and the alimentary tract content. The dry matter content in whole amphipods was similar to that in fish tissues, but macroalgae were more hydrated. Ash in the dry matter of the food items was much higher than in fish tissues. In contrast, carbon, nitrogen, and sulfur levels in the dry matter of the three food items were lower than in fish tissues, and, consequently, the caloric value of the food dry matter was inferior. In comparison with fish muscle, the C/N ratio was elevated in the food items, especially in the phaeophyte *H. grandifolius*. The food items were low in lipids and especially poor in protein; the mean lipid level in fish muscle was higher by factors of 1.6, 2.1 and 4.2 than lipid level in Amphipoda, *C. racovitzae* and *H. grandifolius*, respectively, and protein levels were higher by factors of 2.0, 3.3 and 7.9, respectively. A high percentage of undetermined chemical compounds was found in food organisms, especially in macroalgae *H. grandifolius* (Table 3).

Levels of nitrogen and sulfur (and, consequently, of protein) were higher in both muscle and liver of *L. nudifrons* than in the respective tissues of *N. coriiceps* (Table 3).

Chemical composition of hist muscle, neer and rood nems. Inter-specific comparisons									
	Muscle			Liver			Food		
Materials	N. coriiceps	L. nudifrons	t-test	N. coriiceps	L. nudifrons	t-test	Amphipoda	C. racovitzae	H. grandifolius
materialo	Mean ± SD	Mean ± SD	Р	Mean ± SD	Mean ± SD	Р	Mean	Mean	Mean
D.m.	19.2 ± 1.6 ≈	$18.3 \pm 2.2$	NS	28.1 ± 4.2 >	$24.0 \pm 1.4$	0.001	22.4	8.1	13.9
(% w.w.) ASH (% d.w)	$6.3\pm0.4\approx$	$6.2 \pm 0.4$	NS	5.8 ± 1.8 <	$7.9 \pm 0.4$	0.000	34.3	46.5	26.5
N (% d.w)	$13.6 \pm 0.7 <$	$14.6\pm0.5$	0.000	$9.5 \pm 2.1 <$	$11.7\pm0.6$	0.000	6.8	4.3	1.8
C (% d.w)	$49.3\pm1.6\approx$	$48.2 \pm 1.5$	NS	$53.9 \pm 3.4 >$	$49.2\pm1.1$	0.000	36.3	25.4	34.2
H (% d.w)	8.1 ± 0.2 ≈	$8.0 \pm 0.1$	NS	$8.6 \pm 0.8 >$	$7.4 \pm 0.1$	0.000	5.3	3.8	4.7
S (% d.w)	$1.2 \pm 0.1 <$	$1.5 \pm 0.1$	0.000	$1.0 \pm 0.3 <$	$1.3 \pm 0.1$	0.002	0.7	0.7	0.6
O (% d.w)	21.5 ± 1.3 ≈	$21.5 \pm 1.9$	NS	21.1 ± 3.8 ≈	$22.5\pm0.8$	0.143	16.7	19.3	32.2
CV (J mg <sup>-1</sup>	$26.9\pm0.7\approx$	$26.5\pm0.6$	NS	$29.1{\pm}~2.0>$	$26.1{\pm}0.6$	0.000	19.0	13.5	17.6
d.w.)									
C/N	3.6 ± 0.2 >	$3.3 \pm 0.1$	0.000	$6.1\pm 2.0 >$	$4.2\pm 0.3$	0.001	5.4	5.9	19.4
P (% d.w)	$78.6 \pm 3.8 <$	$84.3\pm2.6$	0.000	55.0 ±12.1<	$67.6 \pm 3.3$	0.000	39.1	24.8	10.2
L (% d.w)	$6.1\pm1.1>$	$4.8 \pm 0.3$	0.000	$23.0\pm2.0>$	$19.6 \pm 3.2$	0.010	3.1	2.4	1.2
"Deficit"* (% d.w)	$9.1\pm3.6>$	$4.8\pm2.6$	0.002	16.2 ±12.9 >	5.0± 3.8	0.002	23.5	26.3	62.1

Chemical composition of fish muscle, liver and food items; inter-specific comparisons

>, <,  $\approx$  and NS as in Table 2; \*Deficit in the tally computed from: 100 - (ASH + P + L).

In contrast, more lipids were found in the tissues of *N. coriiceps* in which a higher C/N ratio was also found.

# INTERRELATIONSHIPS BETWEEN MATTER PROPERTIES AND FISH ATTRIBUTES

Females of *N. coriiceps* were longer (*t*-test, P = 0.024) and heavier (P = 0.013) than males, the female liver weighed more (P = 0.005), and the hepatosomatic index was higher in females than in males (P = 0.023). No sex-induced difference between condition factors in females and males was found. The percentage of sulfur in male muscle dry matter was higher than that in female muscle (P = 0.017). The remaining *N. coriiceps* muscle chemical compounds as well as all liver chemical compounds were independent of sex.

The relationship between the percentage of sulfur in fish tissue dry matter and total body weight was negative and significant both at the intraspecific and interspecific levels (Fig. 1). Strong positive correlations between sulfur content and nitrogen percentages were found in all the tissues (*N. coriiceps* muscle r = 0.691, P = 0.001; *N. coriiceps* liver r = 0.942, P < 0.0005; *L. nudifrons* muscle r = 0.848, P = 0.002; *L. nudifrons* liver r = 0.700, P = 0.024).

TABLE 3



Fig. 1. Size dependence of sulfur content in dry matter of fish tissue. • N. coriiceps muscle, r = -0.800, n = 19, P < 0.0005; • N. coriiceps liver, r = -0.591, n = 19, P = 0.008; • L. nudifrons muscle, r = -0.653, n = 10, P = 0.041;  $\Box - L$ . nudifrons liver, r = 0.232, n = 10, P = 0.518.

## DISCUSSION

The majority of dry matter fish tissues was comprised of protein, lipids, and minerals. The remaining undetermined part (deficit in the tally, 4.8–16.2% of dry matter, Table 2) can be ascribed mostly to carbohydrates and measurement error. The unidentified fraction of dry matter was higher in amphipods and macroalgae than in fish tissues. In amphipods, chitin and the content of the alimentary tract probably contribute to this fraction.

The chemical composition of fish muscle dry matter measured in the present paper ((7) in Table 4) was similar to data reported earlier for Antarctic benthic fishes ((1) – (6) in Table 4), irrespective of different methods and periods related to the annual reproductive cycle, which may generate variability (Love 1970, Casaux et al. 1995). According to the classification proposed by Kołakowska and Kołakowski (2000) for the muscle of boreal fish (Table 4), Antarctic benthic species are lean fish. They are low-fat. Their dry matter is high in protein and minerals, and their fresh matter is highly hydrated. Within the sequence from lean fish to very fatty fish, lipids

of fish muscle and food (recomputed data)									
Material	Dry Matter	Ash	Protein	Lipids	No. of spp.	Source*			
Muscle: benthic fish									
Gobionotothen gibberifrons, An	19.5	5.6	86.9	5.6	1	(1)			
G. gibberifrons, An	20.2	6.4	91.6	4.0	1	(2)			
G. gibberifrons, An	20.2	5.6	88.9	4.2	1	(3)			
Chaenocephalus aceratus, An	18.8	6.4	92.0	4.8	1	(2)			
Champsocephalus gunnari, An	19.0	6.3	89.5	7.9	1	(2)			
Notothenia rossii, An	20.0	5.9	77.7	7.6	1	(4)			
Lepidonotethon squamifrons, An	16.7	6.6	88.0	3.0	1	(1)			
N. coriiceps, An	21.6	6.5	88.4	8.8	1	(2)			
N. coriiceps, An	19.0	5.6	86.5	5.4	1	(5) (6)			
N. coriiceps, An	19.2	6.3	78.6	6.1	1	(7)			
L. nudifrons, An	18.3	6.2	84.3	4.8	1	(7)			
	Mu	ıscle: classi	fication						
Lean fish, B	21.6	6.9	88.0	4.6	7	(8)			
Fairly fatty fish, B	25.0	5.6	74.0	20.0	4	(8)			
Fatty fish, B	30.8	4.5	61.0	34.0	7	(8)			
Very fatty fish, B	36.7	3.3	42.0	54.4	2	(8)			
Fish food									
Macroalgae, Ar	-	16.3	-	3.9	7	(9)			
Macroalgae, An	17.0	22.9	15.5	-	15	(10)			
Macroalgae, An	11.0	36.5	17.5	1.8	2	(7)			
Amphipods, Ar	-	22.6	-	10.9	14	(9)			
Amphipods, An	25.0	28.5	39.0	14.5	1	(11)			
Amphipods, An	22.4	34.3	39.1	3.1	4	(7)			

Comparison of dry matter (% wet weight) and proximate composition (% dry weight) of fish muscle and food (recomputed data)

An – Antarctic species; Ar – Arctic species; B – boreal species.

Phaeophyta and Rhodophyta, Paramoera walkeri.

\*Sources: (1) – Bykov (1972); (2) – Oehlenschläger (1991); (3) - Márquez et al. (1995); (4) - Márquez et al. (1996); (5) - Mazzotta et al. (1993); (6) - Casaux et al. (1995); (7) – present paper; (8) - Kołakowska and Kołakowski (2000); (9) -Wołowicz and Szaniawska (1986); (10) – Zieliński (1988); (11) - Rakusa-Suszczewski and Dominas (1974).

increase dramatically, while water decreases in the muscle of 20 boreal fish species (data by Kołakowska and Kołakowski 2000 in Table 4); the same was found for the whole body matter of 17 species (Schreckenbach et al. 2001).

The chemical composition of food organisms found in the present study ((7) in Table 4) did not differ greatly from the data reported earlier for polar macroalgae ((9), (10) in Table 4) and whole amphipods ((9), (11) in Table 4), with the exception of the lipids level in amphipods. To summarize, whole amphipods are poor in protein and high in minerals, while macroalgae present a combination of low lipids, very low protein, high mineral content in dry matter, and high moisture. Thus, the caloric values of fresh biomass (kJ g<sup>-1</sup>) were 4.3 for amphipods and only 1.8 in macroalgae (mean values, present work). No empty stomachs were found among the *N. coriiceps* sam-

**TABLE 4** 

ples, and the stomachs appeared to be filled with food to near capacity (author's observations). In contrast, a high percentage (42-48%) of empty stomachs was found in Greenland halibut *Reinhardtius hippoglossus* in 1981, 1982 and 1984 (Bowering and Lilly 1999). The annual, mean stomach fullness in *N. coriiceps* (weight of stomach + food as the percentage of total body weight) was 5.0% (95% c.i. 4.7-5.3%) (recomputed raw numerical data from Kamler 2002). Very low stomach fullness values ( $100 \times$  stomach content weight × fish weight<sup>-1</sup>) were reported for the arctic fish *Salvelinus alpinus* 0.09-0.17% (Moore and Moore 1974). The examination of stomach fullness showed that *N. coriiceps* continued to feed during winter (Linkowski et al. 1983, Ashford and White 1995, Kamler 2002). Thus, *N. coriiceps* appears to compensate for the low nutritive value of amphipods and macroalgae by high food intake. Only large *N. coriiceps* individuals are piscivorous (Casaux et al. 1990, Kamler et al. 2001). They preyed on *L. nudifrons*, which are rich in protein (Table 3) and whose lipids are rich in highly unsaturated fatty acids (Kamler et al. 2001).

No sex-induced differences were reported for muscle water and lipid, liver lipid (Mazzotta et al. 1993), or muscle protein and ash (Casaux et al. 1995) in *N. coriiceps* collected over the course of a whole year from Potter Cove, which is in the close vicinity of Admiralty Bay. Similarly, in the present paper no differences in the chemical composition between *N. coriiceps* male and female muscle or between male and female liver were found. The only exception was the concentration of sulfur. In *N. coriiceps* male muscle dry matter, sulfur was higher than in that of females. Sulfur was also higher in both tissues of *L. nudifrons* than in the respective tissues of *N. coriiceps*. These are probably neither sex-induced nor species-specific differences, but simply reflect differences in size. Indeed, levels of sulfur in tissues were negatively related to fish size (Fig. 1). Sulfur and nitrogen were inter-correlated strongly and positively in all the tissues studied in the present paper, because in muscle and liver most sulfate is derived from the sulphur-containing amino acids methionine and cysteine (review in Love 1970). A reduction of various vital rates per unit body weight with increasing size is a common phenomenon.

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## STRESZCZENIE

### SKŁAD CHEMICZNY MIĘŚNI, WĄTROBY I POKARMU DWÓCH GATUNKÓW RYB ANTARKTYCZNYCH Z RODZINY NOTOTHENIIDAE

Badano zawartość głównych pierwiastków (C, H, N i S) i głównych składników (uwodnienie, popiół, białko i lipidy) w tkankach (mięśnie i wątroba) i pokarmie (całe Amphipoda i makroglony) dwóch bentonicznych ryb antarktycznych: *Notothenia coriiceps* i *Lepidonotothen nudifrons*. W mięśniach stwierdzono kombinację wysokich zawartości wody (odpowiednio 80,8 i 81,7%), popiołu (6,3 i 6,2% suchej materii) i białek (78,6 i 84,3% s.m.) oraz niskiej zawartości tłuszczu (6,1 i 4,8% suchej materii) (tabela 3); taka kombinacja jest typowa dla antarktycznych ryb przydennych. Wśród składników pokarmowych – Amphipoda obfitowały w składniki mineralne (34,3% s.m.) i były ubogie w białko (39,1% s.m.), zaś w makroglonach wysokiemu uwodnieniu mokrej materii (89,0%) towarzyszyły wysoka zawartość popiołu (36,5% s.m.), bardzo niska zawartość białka (17,5% s.m.) i niska zawartość lipidów (1,8% s.m.) (tabela 3). Wydaje się, że niska wartość odżywcza pokarmu jest rekompensowana przez jego wysokie spożycie. Poziom siarki w tkankach był ujemnie skorelowany z wielkością ryb (rys. 1). Pozytywne, silne wzajemne korelacje siarki i azotu stwierdzone w tkankach można objaśnić tym, że w mięśniach i wątrobie większość siarki występuje w aminokwasach.

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