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Effects of starvation on the biochemical composition of blood and body tissue in the Antarctic fish *Notothenia coriiceps* (Richardson, 1844) and excreted metabolic products

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Abstract: The study was carried out at the Polish Academy of Science H. Arctowski Polar Station during the 29th Antarctic Expedition in 2005. The experiment involved 18 individual *Notothenia coriiceps* caught in Admiralty Bay. Prior to experimentation, blood was sampled from 12 individuals (six females and six males) and assayed for protein, glucose, triacylglycerol, cholesterol plus cholesterol-HDL fraction levels. Body tissue was also analysed for approximate biochemical composition. Six individuals (four females and two males) were placed in a 400-dm^3 aquarium of constantly aerated seawater at 0.0±0.5°C and starved for 52 days, from mid-April to early June. At the end of the experimental period, blood and body were re-assayed. Starving resulted in body weight reduction and a decrease in blood crude protein content of both males and females. Both males and females showed a significant ($P \leq 0.05$) increase in glucose levels. Triacylglycerol content was reduced—significantly ($P \leq 0.05$) in females and highly significantly ($P \leq 0.01$) (and it is $I \leq 0.01$) in males—and was accompanied by a reduction in total cholesterol. On the other hand, HDL-cholesterol levels increased significantly ($P \leq 0.05$) both in males and females. The seawater in which the fish were maintained was also assayed for various chemicals. Nitrogen and phosphorus levels were elevated relative to those in clean seawater.

key words: Antarctic fish, *Notothenia coriiceps*, starving, blood and body biochemical composition, biogenic

Introduction

Under natural conditions, numerous fish species endure long periods of starvation, associated mainly with seasonal changes in food availability, spawning migrations, preparation for spawning or seasonal changes in water temperature. Due to the high stability of Antarctic water temperatures, feeding activity in Antarctic fish depends principally on ice cover and photoperiod (Johnston, 1993). The metabolism of Antarctic fish is also affected by tides in the inshore zone and light transmission through the water column (Vru-

blevsky, 1984). Reduced energy requirements due to low metabolic rates have also been demonstrated by starvation experiments with *Trematomus eulepidotus* (Wöhrmann, 1998).

The present study aimed at determining the effects of starvation on the condition of the Antarctic fish *Notothenia coriiceps* via changes in body composition, the level of selected blood components and on excretion of nitrogen and phosphorus compounds to the external milieu.

Materials and methods

The study was carried out at the Polish Academy of Sciences' H. Arctowski Polar Station during the 29th Antarctic Expedition of 2005. The experiment involved 18 individual *Notothenia coriiceps* caught in Admiralty Bay. Prior to the experiments, blood was drawn from 12 individuals (six females of 1052±300 g and six males of 645±128 g mean individual weight). Body composition was also determined by appropriate assays. Six individuals (four females of 1670±200 g and two males of 650±60 g mean individual weight) were placed in a 400-dm^3 aquarium of constantly aerated seawater at 0.0±0.5°C. The fish were kept without food in the aquarium for 52 days, from mid-April until early June. On termination of the experiment, the blood and body of the starved fish were reassayed. Fish were weighed at the start and at 10-day intervals during the experiment.

Blood samples were drawn from caudal vessels and centrifuged at 3500 rpm. Serum was kept at −24°C in polypropylene vials. Each series of samples was analysed simultaneously.

Blood serum was used to determine levels of:

- Crude protein (Biuret method, spectrophotometrically, using POCh (Gliwice, Poland) reagents),
- Glucose (enzymatic method, spectrophotometrically, using a diagnostic kit from BioMerieux (Lyon, France)),
- Triacylglycerols (enzymatic method, spectrophotometrically, using a BioMerieux (Lyon, France) diagnostic kit),
- Total cholesterol and HDL-cholesterol fraction (enzymatic method, spectrophotometrically, using a BioMerieux (Lyon, France) diagnostic kit).

Concentrations (%) of the following components were determined in the fish body (beheaded, simply cut and gutted) by the methods of Podeszewski and Stodolnik (1973):

- Crude protein (Kjeldahl method),
- Crude fat (Soxhlet method),
- Dry mass (drying at 105°C for 12 h),
- Ash (combustion at 550°C for 10 h).

Data were subjected to statistical treatment involving one-way analysis of variance (ANOVA) and Tukey's test. Analyses were performed using STATISTICA for Windows software (StatSoft Inc.,1995).

During the experimental period, the tank water was assayed for specific chemicals. The water was changed on day 4, 10, 15, 25, 35 and 45 of the starvation experiment. Water samples were analyzed 24 h after the aquarium had been filled with fresh seawater to determine levels of total nitrogen, nitrite, nitrate and ammonia nitrogen, and reactive and total phosphorus. The assays involved colorimetric methods described in Standard

Methods (1995). Absorbance was measured, at the recommended wavelengths, in a UV-VIS SPECOL-1100 spectrophotometer (Carl Zeiss, Jena, Germany). Nitrite levels were determined using the sulphanyl acid technique (λ=543 nm). Nitrate was assayed as nitrites, after reduction to nitrites in a Cu-Cd column, using the sulphanyl acid technique (λ=543 nm). Ammonia was determined with indophenol blue (λ=630 nm). Total nitrogen was determined, after mineralisation with hypersulphate, as nitrates. The organic nitrogen content was calculated from the difference between total and mineral nitrogen (sum of nitrite, nitrate and ammonia nitrogen). Reactive phosphorus was determined using the molybdenate technique, with ascorbic acid as a reducing agent (λ=882 nm); total phosphorus being assayed, after mineralisation with potassium hypersulphate, as reactive phosphorus. The difference between the two phosphorus forms gave the organic phosphorus content. Water pH, salinity and dissolved oxygen content were measured with a HANNA Instruments HI 9025 pH meter, a WTW FL197 conductometer and a WTW Oxi 197 oxymeter, respectively.

Results

Starvation was found to have a substantial affect on concentrations of blood biochemical parameters (Table 1). Blood crude protein content, in both females and males, did not change significantly during starvation. However, a significant ($P \leq 0.05$) increase in glucose content, in both females and males, was observed. Triacylglycerol content decreased, significantly ($P \leq 0.05$) in females and very significantly ($P \leq 0.01$) in males. The decrease was accompanied by a reduction in total cholesterol content, significant ($P \leq 0.05$) in females and very significant ($P \leq 0.01$) in males. HDL-cholesterol significantly ($P \leq 0.05$) increased in both females and males.

Starvation also resulted in changes in body weight and chemical composition (Table 2)—both body weight and lipid levels decreased. No significant changes were detected in dry body mass or protein levels, in both males and females.

Chemical analyses of seawater showed the nitrogen and phosphorus forms increased 24 h after the tank water had been changed. A particularly large increase was recorded

Table 1. Blood serum component levels in *Notothenia coriiceps* before and after the starvation (χ±SEM).

Component ($mg \cdot dl^{-1}$)	Starvation			
	0 days		52 days	
	Female (n=6)	Male (n=6)	Female (n=4)	Male (n=2)
Protein	58.18± 3.22	64.95± 4.26	54.44± 1.61	53.92±10.62
Glucose	68.32±12.84	110.28±13.24	481.92± 143.31*	368.88±73.73*
Triacylglycerols	589.81±72.15	622.30±61.76	295.74± 76.83*	196.22±36.89**
Total cholesterol	347.99±29.00	281.84±19.50	207.96± 8.28**	209.68±34.69*
HDL-cholesterol	222.06±11.85	176.79±11.21	298.28± 10.46*	249.27± 1.69*

*Significant at $P \leq 0.05$.

**Significant at $P \leq 0.01$.

Table 2. Body chemical composition and mean individual weight of *Notothenia coriiceps* before and after starvation (χ±SD).

	Starvation			
	0 days		52 days	
	Female (*n*=6)	Male (*n*=6)	Female (*n*=4)	Male (*n*=2)
Dry matter (%)	21.57±0.07	22.42±0.21	23.73±1.45	23.75±0.30
Crude protein (%)	17.96±1.04	18.12±0.77	18.45±0.86	18.98±0.59
Lipids (%)	2.14±0.12	2.46±0.06	1.72±0.17	2.15±0.07
Ash (%)	1.43±0.02	1.55±0.10	1.88±0.02	1.53±0.03
Mean individual weight (g)	1670±200	650±60	1520±188	550±43
Mean weight of the beheaded and gutted body (g)	1170±140	460±45	1065±132	390±35

No significant difference.

Table 3. Nitrogen levels (mgN·dm^{-3}) in tank water and seawater from Admiralty Bay.

No.	Day of starvation	NO_2^--N	NO_3^--N	NH_4^+-N	Mineral-N	Organic-N	Total-N
1	1	0.007	0.393	0.877	1.277	0.457	1.734
2	4	0.007	0.399	0.728	1.134	0.381	1.515
3	10	0.006	0.391	0.651	1.048	0.321	1.369
4	15	0.006	0.402	0.607	1.015	0.258	1.273
5	25	0.007	0.418	0.490	0.915	0.269	1.184
6	35	0.006	0.396	0.545	0.947	0.203	1.150
7	45	0.006	0.394	0.460	0.860	0.199	1.059
Average (*n*=7)		0.006	0.399	0.623	1.028	0.298	1.326
Seawater control (*n*=7)		0.005	0.376	0.037	0.418	0.126	0.544

during the first day of starvation, the highest increase (more than 20-fold) involving ammonia nitrogen. Mineral, organic and total nitrogen levels almost doubled, a 3-fold increase being observed in the phosphorus forms assayed. It was only nitrate and nitrite nitrogen concentrations that showed a slight increase (Tables 3 and 4). As the experiment proceeded, the analysed water samples showed a gradual decrease in the nitrogen and phosphorus excreted by the fish.

The nitrogen cycle in the analysed water samples was dominated by mineral nitrogen over organic nitrogen. The former was dominated by ammonia nitrogen (Table 3). The overall mean concentration of this nitrogen form was almost 16 times higher (0.586 mgN-NH_4^+/dm^3) than typical levels in Admiralty Bay. Concentrations of mineral, organic and total nitrogen were, on the average, ~1.4 times higher than those recorded in freshly collected seawater (Table 3).

Higher concentrations of reactive phosphorus were recorded compared to organic phosphorus—the respective mean concentrations being 0.162 and 0.069 mgP/dm^3 (Table 4). Similarly to nitrogen, mean concentrations in the tank water 24 h after it had been changed were 1.1 (organic phosphorus) to 1.5 (reactive phosphorus) times higher than the

Table 4. Phosphorus and selected chemicals levels in tank water and seawater from Admiralty Bay.

No.	Day of starvation	Reactive-P	Organic-P	Total-P	Salinity	pH	Per cent oxygenation
		$mgP \cdot dm^{-3}$			PSU		$\%O_2$
1	1	0.426	0.235	0.661	34.6	7.70	98
2	4	0.293	0.131	0.424	34.6	7.86	96
3	10	0.276	0.116	0.392	34.4	8.01	92
4	15	0.232	0.121	0.353	34.5	7.90	93
5	25	0.215	0.101	0.316	34.1	7.95	90
6	35	0.213	0.102	0.315	34.2	8.00	96
7	45	0.211	0.087	0.298	34.3	7.96	95
Average (n=7)		0.267	0.128	0.394	34.4	7.91	94.3
Seawater control (n=7)		0.105	0.059	0.164	34.4	8.28	100

corresponding concentrations in the freshly collected seawater. Water pH decreased by an average of 0.37 and, despite continuous aeration, the oxygen concentration decreased by an average of 5.7% 24 h after a water change (Table 4).

Discussion

Antarctic fish alternate between the types of food they ingest and show changes in proteolytic enzymes activity, depending on temperature and food type (Rakusa-Suszczewski and Piasek, 1973; Barrera-Oro, 2002). During the Antarctic winter, the fish may cease feeding for some time. The cessation is not related to any difficulty with access to food resources or the lack thereof, but is an effect of temperature reduction, metabolic slow-down and decreased appetite. In addition to winter appetite suppression, *Notothenia coriiceps* shows a reduction in the activity of some enzymes and a decrease in liver triacylglycerol content, compared with the Antarctic summer (Johnston, 1993).

During the Antarctic summer *Notothenia coriiceps* in Admiralty Bay feeds mainly on amphipods, macroalgae and small fish. The experimental starvation period of *Notothenia coriiceps* resulted in changes in the concentration of certain blood biochemical parameters. As starvation proceeded, blood serum of both females and males showed a significant increase in glucose concentration. Hyperglycaemia in fish, as frequently in higher vertebrates, is an effect of some stimuli, so-called stressors (*e.g.* a change in water temperature or type of diet) acting upon the fish body. The fish metabolic system shows a stressor-induced intensification of secretion of many hormones, *e.g.* catecholamines (adrenaline and noradrenaline), which accelerate glycogen breakdown in the liver and muscles by activation of glycogen phosphorylase and cortisol; in addition to other effects, cortisol increases gluconeogenesis (Pickering, 1981; Van-Raaij *et al.*, 1996). By keeping notothenioid fish for 5–6 weeks in water heated to 4°C, Lowe and Davison (2005) observed an increase in the blood glucose content, which was mainly a cortisol-induced effect with little contribution from adrenaline or noradrenaline.

It is known, however, that prolonged starvation in fish leads to hypoglycaemia (De Silva and Anderson, 1995); therefore, it appears that the increased glucose level in blood was related to the fish being ready to spawn (the fish were already dripping). Glucose content in fish blood increases during vitellogenesis and peaks during spawning (Hoar *et al*., 1983). Svoboda *et al*. (2001) observed a significant increase in blood glucose in female and male Tench kept in ponds for 2 months and ready to spawn, compared to initial levels.

In the present study, starvation was terminated in early June. The species spawns in May/June (Kock and Kellerman, 1991), so it is assumed that blood glucose was affected by physiology (readiness to spawn) rather than the lack of food and a possibly associated stress. *Notothenia coriiceps* shows a relatively low body fat content (Kołakowska, 1987, 1988; Kamler *et al*., 2001). The prolonged starvation affected lipid metabolism, as shown by the significant differences in blood lipid components and differences in body lipid levels of the experimental fish.

A decrease in blood triacylglycerol concentration, significant in females and highly significant in males, was observed. The significant reduction in total cholesterol, combined with the significant increase in the HDL-cholesterol, provides additional evidence of an altered metabolism in *N. coriiceps* subjected to starvation. A similar reduction in triacylglycerol and total cholesterol concentrations, accompanied by an HDL-cholesterol increase, was observed in starved Carp (Friedrich and Stepanowska, 2001).

Prolonged starvation also induced significant changes in fish body chemical composition (Table 2). Body lipid levels decrease as a direct effects of food shortage (Heming and Paleczny, 1987; Crockett and Sidell, 1990; Sidell, 1991).

On the other hand, no reduction in protein level was observed. On the contrary, the body protein concentration was even found to have increased. However, it is assumed that the observed tissue protein increase is an artefact produced by a change in fish body dry mass, not accompanied by any loss of tissue protein. A similar effect was observed during a 12-week starvation in Carp. Increased protein levels, accompanied by a changing proportion of dry mass, were observed as early as week 4 in starved Carp (Friedrich and Stepanowska, 2001).

Productivity of Antarctic waters depends on temperature, light intensity, water mass dynamics and nutrient contents. The highest concentrations of nitrate nitrogen, reactive phosphorus and silica in the Southern Ocean are not correlated with low phytoplankton production. Oxidised nitrogen is the dominant form of nitrogen; hence primary production is limited mostly by the availability of reduced nitrogen (ammonia nitrogen) (Konnov and Borogkin, 1993). This study shows the dominance, typical of the Southern Ocean, of nitrate nitrogen over ammonia nitrogen. On the other hand, the study showed a substantial increase in ammonia nitrogen concentration, caused by the fish excreting metabolic products and undigested food; a slight increase in nitrate nitrogen was also recorded. Twenty-four hours after the water in the tank was changed, the average concentration of ammonia nitrogen was more than 25 times that of nitrate nitrogen (Table 3). This significant effect of fish was not detected with phosphorus level in the water. A high phosphorus increase was observed during the initial period of starvation, when the tank water was most intensely contaminated with undigested food released by the fish (Table 4). After 4 days, the concentration of all phosphorus forms dropped to levels, which were

average for the entire study period, but were slightly higher than those reported by Pęcherzewski (1980) for Admiralty Bay. As shown by Pęcherzewski (1980), Samp (1980), Tokarczyk (1986) and Lipski (1987), Admiralty Bay water, particularly in the coastal zone, is characterised by slightly higher nutrient levels, as well as particulate and dissolved organic matter, compared to the open water of the Southern Ocean. Decomposition of algal material and freshwater runoff, enriched by guano leached from penguin rookeries, are important sources of nitrogen and phosphorus in the area (Juchnowicz-Bierbasz and Rakusa-Suszczewski, 2002; Rakusa-Suszczewski and Nędzarek, 2002; Nędzarek and Rakusa-Suszczewski, 2004). As found by Nędzarek and Rakusa-Suszczewski (2004), decomposition of marine macroalgae results in the release of about 10.5 g nitrogen and 12.9 g phosphorus per 1 kg algal dry weight. Macroalgae cover 30% of the Admiralty Bay bottom; about 1600 tonnes of macroalgal remains are transported by wave action onto the shore and winds move the plant fragments still further inland (Zieliński, 1981). The increase in nutrient concentration in the coastal part of Admiralty Bay is, therefore, mainly a result of organic matter cycling between the sea and land. The effect of excretion of nitrogen and phosphorus by fish on water nitrogen concentrations is insignificant. However, excretion may be important for chemoreception—in finding mates, sending warning signals in the presence of predators or in prey hunting—and there are numerous behaviour-inducing substances, including amino acids, amines, organic acids, lipids, nucleotides, sugars and mineral salts (Carr *et al*., 1996; Zimmer and Butman, 2000). This study demonstrated ammonia nitrogen to be the major nitrogen form excreted by the fish. Ammonia nitrogen is the main component of amino acids, for example. Despite numerous sources of amino acids (excretion by animals and plants, release during decomposition of dead organic matter), their concentration in sea water is usually low (Braven *et al*., 1995). For example, concentrations of major amino acids (alanine, serine, glycine, aspargic acid and glutaminic acids) in the Drake and Bransfield Straits reached values in the order of 10^{-7}–10^{-6} mol·dm^{-3} (Mężykowski, 1982). On the other hand, marine organisms are capable of detecting much lower amino acid levels: the signal-to-background ratio is probably more important than the absolute concentration of a chemoattractant (Handrich and Atema, 1986).

Conclusions

During several weeks of starvation in *Notothenia coriiceps*, reduced blood triacylglycerol and total cholesterol concentrations, and a decrease in body lipid content, were observed. The absence of mortality and the good condition of the fish indicate that *N. coriiceps* can survive long periods of food shortage.

The study showed that concentrations of nitrogen and phosphorus (mainly in the form of ammonia nitrogen and reactive phosphorus) increased as a result of metabolic product excretion. The increased nitrogen and phosphorus concentrations, recorded during starvation, are irrelevant with respect to open-ocean fertility (too high volume and water dynamics), but may be important for chemoreception.

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