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# Comparison of fatty acid composition in nine organs of the sympatric Antarctic teleost fish species *Notothenia coriiceps* and *Notothenia rossii* (Perciformes: Nototheniidae)

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ABSTRACT

Fatty acid (FA) composition of nine organs from two closely related Antarctic fish species, *Notothenia coriiceps* and *Notothenia rossii*, was determined through gas chromatography with flame ionization detection. A data set for each species was obtained using major FA profiles from specimens caught in the sea waters of Admiralty Bay during the summer season. The FA profiles for both species are overall similar, but organ peculiarities have been found, which could reflect metabolic specificities and feeding habits between species. With the exception of liver, the most abundant FA in organs was the $n-3$ polyunsaturated FA. The total $n-6$ polyunsaturated FAs were minor components in all evaluated organs. Palmitic acid was identified as the major saturated FA, whereas oleic acid was the most represented of the monounsaturated FA in almost all assessed organs of both species. The $n-3/n-6$ ratios of all organs were higher than 3.5. Differences in individual FA and FA metabolic profiles of some organs observed between *N. coriiceps* and *N. rossii* suggest specific requirements in the mobilization, transport, incorporation, and/or catabolism of lipids that were reinforced by differences on some FA ratios expressing the activity coefficient of enzymes implicated on the FA pathway flux.


## 1. Introduction

Antarctic marine life is broadly associated with successful metabolic adaptation to a cold environment (Pörtner et al., 2007). Low temperature, higher oxygen solubility in water, and high levels of UV radiation are some of the extreme environmental insults to fish (Karentz et al., 1991; Pörtner et al., 2007). In this respect, fish adaptation is verified at the molecular, cell, tissue, and organ hierarchical organization levels (Karentz et al., 1991; Hagen et al., 2000; Pörtner et al., 2007; Wujcik et al., 2007; Chen et al., 2008). Although most of these adaptive mechanisms are based on gene-encoded strategies, some are acquired from the diet as in the case of lipid intake (Hagen et al., 2000).

The metabolism of lipids in vertebrates is strongly influenced by the overall fatty acid (FA) availability. Although some FAs in fish arise from the *de novo* synthesis of non-lipid carbon sources, the majority of them are directly incorporated from dietary lipids (Morais and Conceicao, 2009). Due to the specificity of metabolic enzymes involved in lipid elongation and desaturation, the absolute amounts of FA families and the proportions of FA in the diet are of physiological relevance (Turchini and Francis, 2009). Hence, dietary lipids not only modulate the balance among the different types of FA incorporated in phospholipids, but also play a major role as a source of metabolic energy (Tocher, 2003). In this respect, FA profiles in organisms differ markedly among fish body parts, in particular when considering their specific metabolic requirements (Bell et al., 2001).

Members of the suborder Notothenioidei dominate the Antarctic ichthyofauna both in terms of number of species and biomass. For this reason most of the current knowledge regarding cold-adapted fish physiology derives from this suborder (Near and Cheng, 2008). However, little is known about the FA profile of several Notothenioid organs. FA composition has a crucial role during local production of lipid mediators, cell membrane functionality, and lipid peroxidation susceptibility (Bell et al., 2001; Tocher, 2003; Peng et al., 2009). The present study focuses on the investigation of the FA composition of nine organs from two endemic and sympatric Antarctic fish *Notothenia coriiceps* (Black rockcod) and *Notothenia rossii* (Marbled rockcod).

*Abbreviations:* ACL, Average chain length; FAME, Fatty acid methyl esters; FA, Fatty acids; GC/FID, Gas chromatography with flame ionization detection; MUFA, Monounsaturated fatty acids; PUFA, Polyunsaturated fatty acids; RMR, Routine metabolic rate; SFA, Saturated fatty acids; UFA, Unsaturated fatty acids; UV, Ultraviolet.

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## 2. Materials and methods

### 2.1. Animals

Adult *N. coriiceps* ($n=3$) and *N. rossii* ($n=3$) were collected by line and hook (about 15–25 m depth) at Admiralty Bay (King George Island, South Shetland Islands, Antarctica Peninsula) in December 2007. The fish were killed by decapitation and the organs dissected out, trimmed of any adhering adipose tissue, washed on phosphate–saline buffer (0.01 M, pH 7.2), and stored in liquid nitrogen. The FA composition was examined in stomach, pyloric caeca, intestine, liver, head kidney, spleen, gonad, heart, and muscle.

### 2.2. Lipid extraction

Approximately 300–500 mg of each frozen organ was weighed into a test tube and 2 mL of methanol was added. The sample was homogenized in a ground glass tissue grinder for 30 s at 4 °C and vortexed. The homogenizer was rinsed in 1 mL of chloroform and the rinsed fluid was combined with the homogenate. The homogenate was filtered and to the volume obtained 1 mL of nanopure water was added. The mixture was vortexed for 30 s, centrifuged at 900 *g* for 3 min, and the methanol layer was discarded. The chloroform layer was transferred to other test tube, and reextracted with Folch solution (chloroform:methanol; 2:1; v:v) following evaporation of the downer phase under nitrogen (Folch et al., 1957). Lipids were saponified in 2 mL of 0.5 M KOH at 37 °C for 2 h and FA methyl esters (FAME) extracted with 2 mL of hexane.

### 2.3. Fatty acid analyses

The hexane solution was filtered (0.45 μm) and injected (1 μL) for the separation of FAME through CG/FID (Shimadzu CG17A, automatic injector AOC20I. Kyoto, Japan) with a Supelco SP2340 [100% poly (biscyanopropyl siloxane) − 60 m × 0.25 mm id × 0.2 μm] column at an initial temperature of 120 °C/5 min followed by a 4 °C/min increase until reaching 240 °C, maintained for 10 min. Helium was used as the carrier gas, at 17 cm/s (0.67 mL/min). The injector and detector temperature were 250 °C and 260 °C, respectively. The sample peaks were identified by the retention times obtained through comparison with standards (Supelco kit FAME Mix).

The relative amount of each FA was quantified by integrating the area under the peak and dividing the result by the total area for all FAs. Eleven FAs that exceeded a minimum of 0.5% total FA were identified and compared between the species. Saturated FAs were calculated as SFA = sum of the percentage of myristic (14:0), palmitic (16:0), and stearic (18:0) acids. Unsaturated FAs were calculated as UFA = sum of the percentages of palmitoleic (16:1, $n-9$), oleic (18:1, $n-9$), elaidic (18:1, $n-9$ trans), linoleic (18:2, $n-6$), alpha-linolenic (18:3, $n-3$), arachidonic (20:4, $n-6$), eicosapentaenoic (20:5, $n-3$), and docosahexaenoic (22:6, $n-3$) acids. Oleic and elaidic acid amounts were compared as a sum in certain analyses since these isomers were not resolved in all GC chromatograms. Monounsaturated FAs were calculated as MUFA = sum of the percentages of palmitoleic, oleic, and elaidic acids. Polyunsaturated FAs $n-3$ were calculated as PUFA $n-3$ = sum of the percentages of linolenic, eicosapentaenoic and docosahexaenoic acids. Polyunsaturated FAs $n-6$ were calculated as PUFA $n-6$ = sum of the percentages of linoleic and arachidonic acids. Some ratios of these FA groups were calculated from the primary data.

The activities of specific enzymes involved in FA biosynthesis were estimated as the product/precursor ratios of the percentages of individual FA. The estimated enzyme activities comprise those of: elongase, calculated as the 18:0/16:0 ratio; delta-5 desaturase, calculated as the 20:4/18:3 ratio; delta-6 desaturase, calculated as the 18:3/18:2 ratio; and delta-9 desaturase, calculated as the 18:1/18:0 ratio (Andersson et al., 1998). The 20:4/18:2 and 22:6/18:3 ratios express the activity coefficient of enzymes in the biosynthetic pathway of arachidonic acid from linoleic acid and docosahexaenoic acid from linolenic acid, respectively.

The unsaturation index was calculated as the sum of the percentage of monoenoic FA × 1, dienoic FA × 2, trienoic FA × 3, tetraenoic FA × 4, pentaenoic FA × 5, and hexaenoic FA × 6 which represents the sensitivity of the organ to oxidative damage. The average chain length (ACL) was obtained as the sum of the percentage of each FA multiplied by its number of carbons and divided by 100.

### 2.4. Statistical analyses

All values are expressed as the mean and standard error of the mean (SEM) as indicated in the tables and graphs. Data were compared with ANOVA to detect differences between the species and PLSD Fischer's test was employed to indicate statistical significance ($p<0.05$). Hierarchical cluster analysis was carried out to underline any correlation between the analyzed FA parameters and the corresponding organ.

## 3. Results and discussion

### 3.1. Stomach, pyloric caeca, and intestine

The FA composition and the calculated FA metabolic profiles of the digestive tract organs of *N. coriiceps* and *N. rossii* are shown in Fig. 1 and Table S1. No significant differences were found between the UFA stomach profiles of each species, even though *N. coriiceps* contained higher MUFA and lower $n-3$ PUFA levels when compared to *N. rossii* ($p<0.05$). In addition, *N. coriiceps* contained a significantly higher 18:0 content (34%) when compared to *N. rossii* ($p<0.05$). This fact led to the higher estimated stomach elongase activity (25%) in *N. coriiceps* when compared to *N. rossii* ($p<0.05$). Although estimating elongase activity as the ratio between individual FAs has shortcomings, direct measurement of enzyme activity is not feasible. Thus, estimated enzyme activities in this case may be useful for understanding FA synthesis on a comparative basis. Studies have shown that the zebrafish elongase activity in transformed yeast was associated to increased levels of MUFA (Agaba et al., 2004). Therefore, the higher stomach MUFA level can be positively correlated with estimated elongase activity on *N. coriiceps* compared to *N. rossii*. In addition, the higher stomach $n-3$ PUFA level in *N. rossii* directly correlates to significantly higher ACL and unsaturation index when compared to *N. coriiceps* ($p<0.05$). The relevance of high levels of FA unsaturation is largely justified due to the extensive literature relating degree of FA unsaturation to intrinsic peroxidation potential of an organ. Further studies are necessary to correlate such data with the possible increase of membrane lipid peroxidation.

Along the proximal intestine of *Notothenia* species, an anatomical specialization consisting of blind-ending sacs termed pyloric caeca aids in the digestion and subsequent absorption of lipid-rich diets (Olsen and Ringo, 1997). There was no significant difference in individual and total SFA, MUFA, $n-6$ PUFA, and FA metabolic profiles of the pyloric caeca between either species ($p>0.05$). However, there was a concomitant significantly higher (8.5%) relative content of 20:5 and a lower (7.8%) relative content of 22:6 in *N. coriiceps* than in *N. rossii* ($p<0.05$). Although the physiological relevance of such findings is not clear, they could reflect specific metabolic attributes or feeding preferences between these Antarctic species. Evidence points to a critical role of the pyloric caeca in lipid metabolism and even biosynthesis of highly unsaturated long-chain FA (Tocher, 2003; Zheng et al., 2005).

Corroborating these data, the anterior segments of the intestine immediately after the pyloric caeca showed a profile of PUFA similar to the pyloric caeca. Hence, there was a significantly higher (24%) relative content of 20:5 and a lower (46%) relative content of 22:6 in

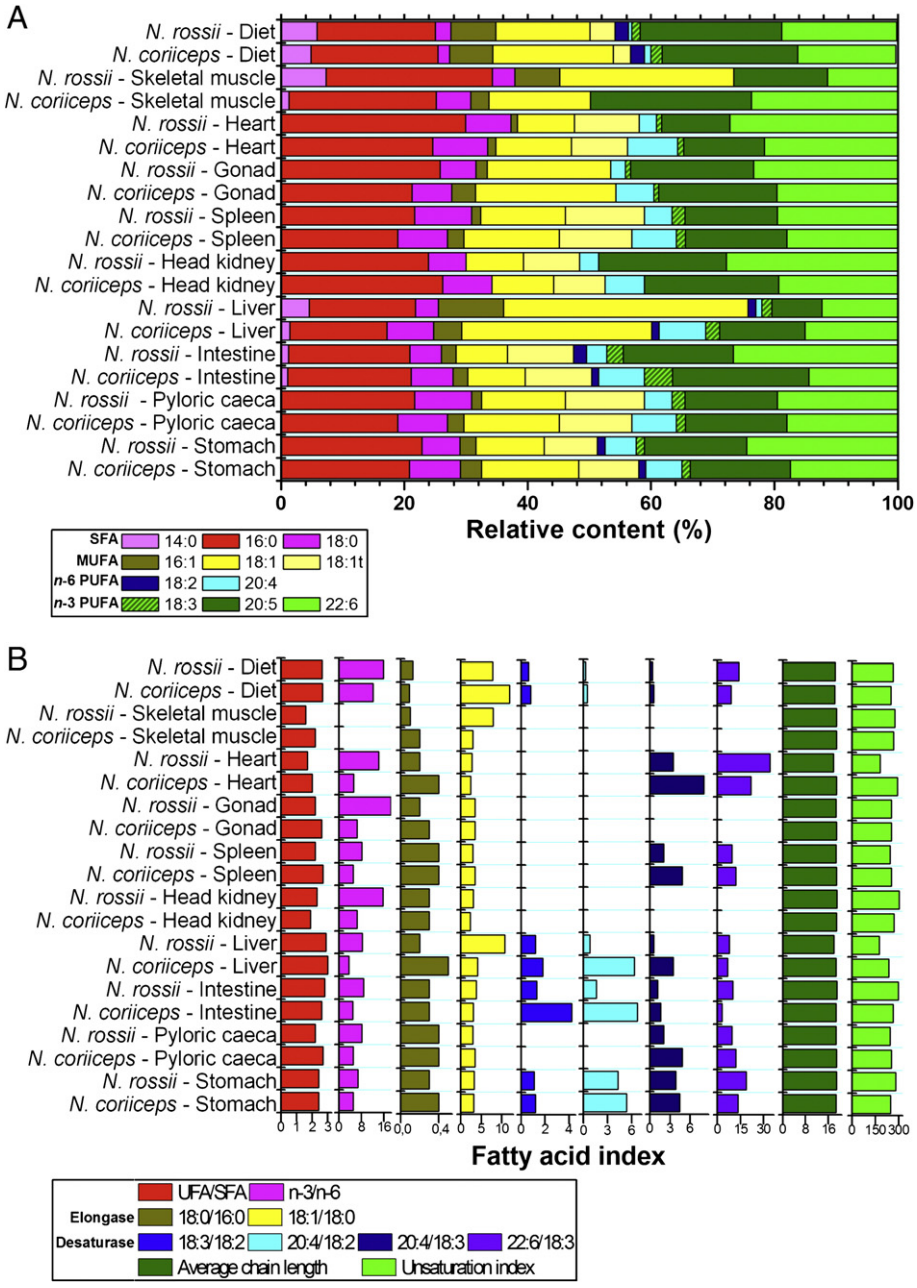


**Fig. 1.** Stacked bar representations of the relative content of individual FA (A) and bar representations of the FA metabolic profiles (B) of *N. coriiceps* and *N. rossii* organs and their diet. Missing bars indicate absent FA or incalculable index.

*N. coriiceps* when compared to *N. rossii* ($p<0.05$). In addition, there was a ~2-fold higher relative FA content of 20:4 in *N. coriiceps* intestine than in *N. rossii* ($p<0.05$). In teleost fish, the anterior intestine is the major site of lipid absorption (Garrido et al., 1993). Since the fish intestines were collected only a few minutes after capture with no fasting period, it is possible that such FA profile could correspond to the products of digestion of the diet recently uptaken by enterocytes. Therefore, the observed differences on the relative levels of PUFA between the species could also reflect different diet preferences, since the species were captured in distinct points of the Admiralty Bay (*N. coriiceps* at Punta Hennequin and *N. rossii* at Punta Plaza).

### 3.2. Liver

The lipid dietary uptake and the *de novo* synthesis of FA in the liver are hallmarks of vertebrate lipid metabolism. FAs are generally intended to oxidation or esterification to either glycerol or cholesterol for the synthesis of triglycerides and cholesterol esters, respectively (Hernandez-Blazquez et al., 2006). Fig. 1 and Table S1 show the liver FA composition of the notothenioid species. The data show that the relative amount of individual FA was similar between the species ($p<0.05$). There was only a ~3-fold higher relative content of 14:0 on *N. rossii* when compared to *N. coriiceps* ($p<0.05$). Since 14:0 constitutes only a minor component of the FA profile, this alteration should not have any physiological relevance. However, the ratio 18:0/16:0 was lower in *N. rossii* compared to *N. coriiceps* ($p<0.05$). These findings could indicate subtle differences in FA chain elongation systems between the species which will require further characterization of elongase gene expression levels and activities. Another hypothesis is that SFA could be selectively incorporated from the diet in *N. rossii* organs requiring higher energy supply.

### 3.3. Head kidney and spleen

Fig. 1 and Table S2 show the FA types and FA metabolic profiles of head kidney and spleen of *N. coriiceps* and *N. rossii*. The composition of FA of Antarctic fish is relatively well characterized in liver and muscle (Hagen et al., 2000; Kamler et al., 2001), but little is known on the FA content of lymphoid organs. The adaptation of lymphoid organs to low temperatures in Antarctic fish species could give insights into how the immune system functions under extreme environmental conditions. Like other vertebrates, these fish have an adaptive immune system driven mainly by leukocytes present in lymphomyeloid organs, such as head kidney and spleen (Romano et al., 2000).

The proportion of 22:6, total $n-3$ PUFA, and $n-3/n-6$ ratio was significantly higher (44%, 18%, and 2.4-fold, respectively) in head kidney of *N. rossii* when compared to *N. coriiceps* ($p<0.05$). There are situations where the combination of these parameters strongly points to an enhanced membrane fluidity associated with cold tolerance in fish (Jobling and Bendiksen, 2003). In addition to altering membrane fluidity, such parameters could alter the immune system functioning of lymphoid organs, particularly with regards to eicosanoids production from $n-3$ PUFA (Bell et al., 1996). Despite the absence of any known comparative study describing the immune response of *N. coriiceps* and *N. rossii* towards the same biotic insult, there is evidence suggesting a prevalence of pathogen infections in *N. coriiceps* (Palm et al., 1998). Moreover, information on the relationship of tissue FA composition to specific infection resistance requires future investigations.

There was no significant difference between individual spleen FA composition in either *N. coriiceps* and *N. rossii* ($p<0.05$). However, significantly higher 20:4/18:3 (2.3-fold) and 22:6/18:3 (29%) ratios were noticeable in *N. coriiceps* when compared to *N. rossii* ($p<0.05$). Interestingly, reports have demonstrated that the production of 22:6 in fish proceeds via elongation and desaturation steps of 18:3 (Buzzi et al., 1996). In agreement with these results, fish skin cells have also been shown to incorporate and metabolize 18:3 by desaturation/elongation pathways in which 20:4 and 20:5 are the major end-products, with lower desaturation activity of 20:5 to 22:6 (Ghioni et al., 1997). However, it is unclear whether these FA ratio changes could have some physiological importance in *N. coriiceps* spleen when compared to *N. rossii*, *e.g.* stimulating eicosanoids production.

### 3.4. Gonad

FA composition obtained from the gonads of *N. coriiceps* and *N. rossii* is shown in Fig. 1 and Table S2. Since FA compositions of fish testes and ovaries are described as similar (Hiratsuka et al., 2004) and all presently investigated animals had immature gonads, these organs were evaluated as a pool. There was a 22% and 19% higher amount of 16:0 and 22:6, respectively, in *N. rossii* gonad when compared to *N. coriiceps* ($p<0.05$). Clear differences between reproductive habits of *N. coriiceps* and *N. rossii* have been compiled from the available literature (Kock and Kellermann, 1991). Among several distinguishing aspects, *N. rossii* has a higher gonadosomatic index, lower estimated time of incubation of the eggs, and shorter pelagic phase than *N. coriiceps* (Kock and Kellermann, 1991). Since fish initial development stages are strictly dependent on the FA availability (Morais and Conceicao, 2009), the differences observed for some individual FA between the species may lead to reproductive specific features.

### 3.5. Heart and skeletal muscle

No marked significant differences were observed on heart individual FA of *N. coriiceps* and *N. rossii* (Fig. 1 and Table S2). However, *N. rossii* had a significantly 3.2-fold higher $n-3/n-6$ ratio when compared to *N. coriiceps* ($p<0.05$). Fish tissue $n-3/n-6$ ratio is strongly influenced by diet and the cardiovascular physiology is directly influenced by FA composition (McKenzie et al., 1999). A higher $n-3/n-6$ ratio in tissues is associated with improved tolerance to physiological stresses including cold, live transport, hypoxia, and exhaustive exercise. In this way, fish fed highly on $n-3$ PUFA (high $n-3/n-6$ ratio) had significantly lower routine metabolic rate (RMR) compared to those fed a SFA diet (McKenzie et al., 1999). Therefore, the differences in $n-3/n-6$ ratio in the heart of *N. coriiceps* and *N. rossii* were presumably a result of possible differences in RMR. This assumption is borne out by the fact that a recent data compilation (Makarieva et al., 2008) showed that the normalized RMR of *N. coriiceps* and *N. rossii* are 0.390 and 0.304 W (kg WM)$^{-1}$, respectively. The mechanisms underlying the effects of PUFA on RMR are unknown. However, since aerobic metabolism in fish is primarily fuelled by FA oxidation with a substrate preference for MUFA > SFA > $n-6$ PUFA > $n-3$ PUFA, the lower RMR of *N. rossii* with heart showing high $n-3/n-6$ ratio could be the result of sub-optimal oxidation. This fact is also reinforced by a significantly 2.4-fold higher 20:4/18:3 ratio in *N. coriiceps* when compared to *N. rossii* ($p<0.05$).

With regards to individual FA composition of fish muscle, there was a 5.6-fold and 71% higher relative amount of 14:0 and 18:1, respectively, in *N. rossii* when compared to *N. coriiceps* ($p<0.05$). Significantly higher amounts of 14:0 ($p<0.05$) and higher contents of 18:1 (not significant) were also observed in liver of *N. rossii*. These facts could be related to some liver to muscle lipid flux mechanism.

### 3.6. Diet

To date, several reports demonstrate that the tissue FA profile in fish largely reflects their dietary intake (Garrido et al., 1993; Bell et al., 1996, 2001; McKenzie et al., 1999; Hagen et al., 2000; Kamler et al., 2001; Jobling and Bendiksen, 2003; Peng et al., 2009; Turchini and Francis, 2009). In fact, the FA composition of organs in *N. coriiceps* and *N. rossii* is influenced by the pattern of FA available in the preferable diet. Whereas *N. coriiceps* is an opportunist and generalist omnivorous known to feed on a wide spectrum of food stuff mainly based on salps, algae, amphipods, and isopods, *N. rossii* is a voracious predator of salps, small fish, amphipods, polychaetas, and also krill (Barrera-Oro, 2003). A compilation from the current literature (Supplementary references) of the main FAs present in major diet items according to data described by Barrera-Oro (2003) is shown in Fig. 1 and Table S3. The major difference within species diet is a 9.7 fold higher absolute amount of small fish present in *N. rossii* diet when compared to that of *N. coriiceps*. Due mainly to this fact, a 30% higher $n-3/n-6$ ratio is observed in the diet of *N. rossii* when compared to that of *N. coriiceps*.

### 3.7. Overall comparisons

Fig. 2 illustrates simultaneously the overall data obtained using permuted data matrix maps of hierarchical cluster analyses of FA types and FA metabolic profiles. The principal observation emerging from the comparison of the FA composition of *N. coriiceps* and *N. rossii* is that they slightly differed between species and moderately differed among organs. Almost all organs were characterized by high levels of $n-3$ PUFA, predominantly 22:6 and 20:5, along with substantial levels of MUFA and SFA; minimum to trace levels of $n-6$ PUFA; and an ubiquitous extremely high $n-3/n-6$ ratio (always of more than 4.5) more pronounced in all *N. rossii* organs when compared to *N. coriiceps*. The high amounts of $n-3$ PUFA in organs of the currently studied species are in good agreement with data available in the literature (Kamler et al., 2001). PUFA levels are high in fish from cold regions, whereas SFA and MUFA are generally abundant in fish from temperate and warm regions of the planet. The very low amount of 20:4 found in all organs of both species is related to the low percentage or even lack of one of its precursors, 18:2. Palmitic acid (16:0) was the major SFA, contributing 16–30% of the total FA content for all organs of both species. Oleic acid (18:1) was the most represented of the MUFA, accounting for 8.3–39.6% of total FA for organs of both species.

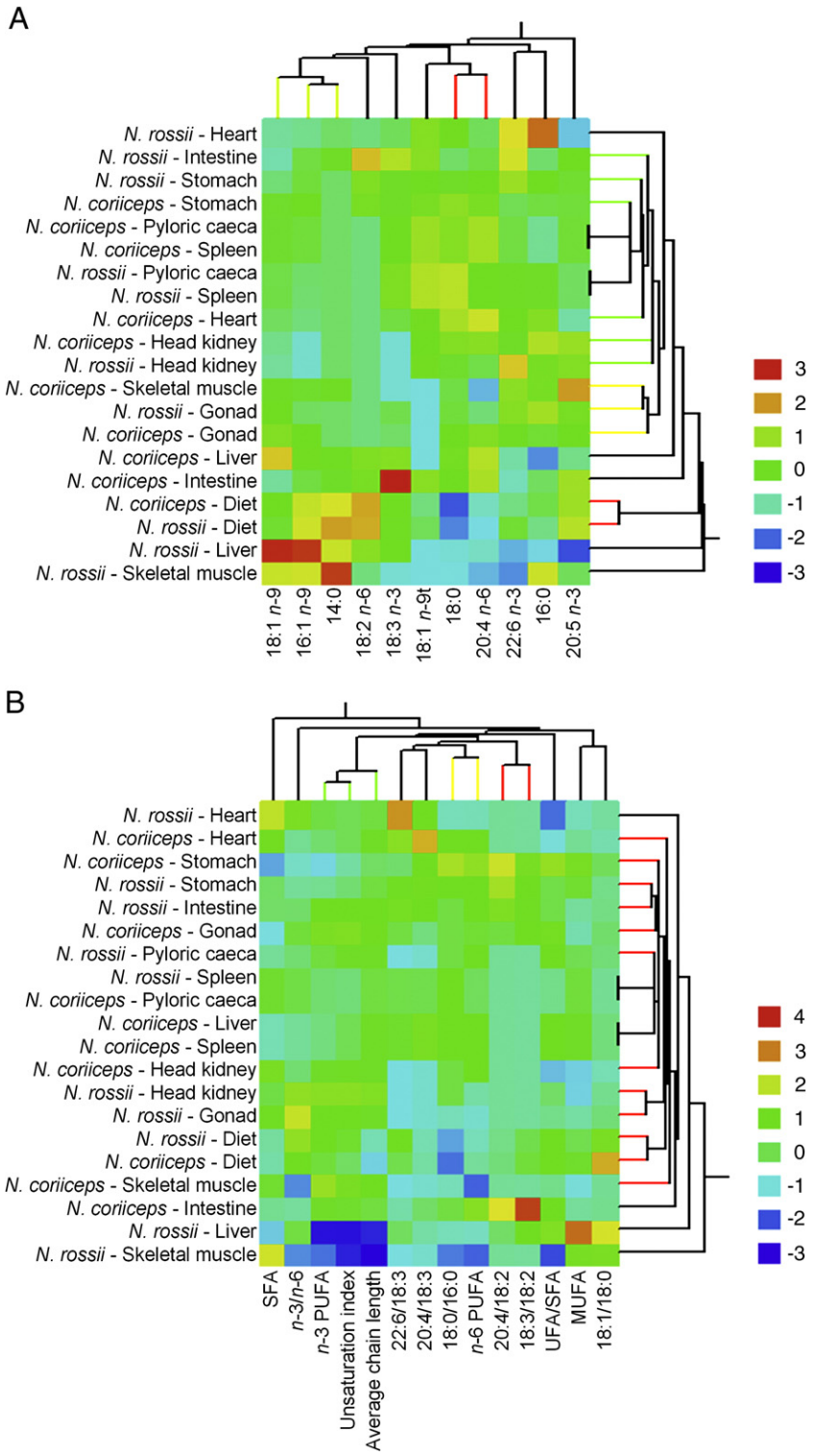


**Fig. 2.** Permuted data matrix map of hierarchical cluster analyses of FA types (A) and FA metabolic profiles (B) of *N. coriiceps* and *N. rossii* organs and diet. Red-orange, green, and blue dots represent a high, moderate, and low relative content/value of each analyzed parameter, respectively. Colour scales represent the normalization of the compared data.

At present we have no direct indication about the mechanism(s) causing the observed differences in FA profile among different *Notothenia* organs. Possibilities include altered rates and/or selectivity in FA uptake, transport, release, oxidation, biosynthesis, and deacylation–reacylation reactions. All these possible explanations may be due to changes in the activity and or expression of the proteins involved on FA pathway flux as indicated by some differences of FA ratios expressing the activity coefficient of enzymes (Fig. S1). Genomic and proteomic investigations of these organs are currently underway and will help clarify some of these hypothesized mechanisms.

In sum, the present study describes a screening of the FA profile of nine vital organs of two abundant Antarctic fish species. New insights into specific organ FA requirements aiming the survival in polar climate adversities were presented and discussed.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi: 10.1016/j.cbpb.2009.10.012.

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