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The impact of seawater warming on fatty acid composition and nutritional quality indices of *Trematomus bernacchii* from the Antarctic region

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ABSTRACT

There is a growing interest in exploiting Antarctic fisheries for human consumption. However, information on how the nutritional qualities of these resources will respond to the predicted seawater warming in the region for the next century is poor. The present research investigates changes in various nutritional indices of dietary importance (e.g. the ratio polyunsaturated to saturated fatty acids, the atherogenicity index, the thrombogenicity index, the hypo-cholesterolemic to hyper-cholesterolemic index, the health-promoting index, the flesh lipid quality and the ratio omega-3 to omega-6 index) by determining the fatty acid composition in muscle of *Trematomus bernacchii* (an Antarctic fish species) in its natural habitat (-1.87 °C) and warmer temperatures (0.0, 1.0, 2.0 °C). Comparison of the estimated nutritional indices at −1.87 °C with those at warmer temperatures revealed that seawater warming caused changes in the nutritional indices in the range of $-12\% < \Delta < 30\%$. The observed changes were not statistically significant and ascribed to biological variability. Therefore, the nutritional values of *T. bernacchii* muscle were preserved after increasing the temperature of its natural habitat by + 4 °C. The present research is the first report describing the nutritional quality indices for an Antarctic fish species and the consequences of seawater warming on the nutritional value of *T. bernacchii*.

1. Introduction

Fish is a rich source of nutrients such as proteins, vitamins, essential minerals, amino acids and polyunsaturated fatty acids (PUFA) and its consumption is widely acknowledged to have a positive impact on human health. Marine fish species contain high levels of the omega-3 PUFA, more specifically eicosapentaenoic acid (20:5n-3; EPA) and docosahexaenoic acid (22:6n-3; DHA). These PUFA are the most important substrates in the synthesis of biologically active anti-inflammatory mediators which play a key beneficial role in inflammation pathologies such as cardiovascular diseases and neurodegenerative disorders (Chitre, Moniri, & Murnane, 2019; Jung, Torrejon, Tighe, & Deckelbaum, 2008). Therefore, an increased consumption of seafood is regarded as an important nutritional strategy to protect human health (Hellberg, DeWitt, & Morrissey, 2012).

Nowadays, due to the benefits of seafood, an especially the n-3 PUFA, the content of fatty acids is one of the most reported nutritional properties of fish. The composition of fatty acids in fish varies considerably between species, for instance, fatty fish (e.g. salmon, tuna, trout, mackerel) contain higher amounts of n-3 fatty acids than lean fish (cod, halibut, bass, flounder) (Cahu, Salen, & De Lorgeril, 2004; Jobling, Leknes, Sæther, & Bendiksen, 2008; Peng, Chen, Shi, & Wang, 2013), and some quantitative indices have been introduced to objectively assess the nutritional quality of different fish species. For example, the PUFA to saturated fatty acids (SFA) ratio (PUFA/SFA) to indicate the risk of cardiovascular diseases and oxidative stress (Chen & Liu, 2020); the atherogenicity index (AI) to indicate the relationship between pro-atherogenic fatty acids (those that promote lipids adhesion to cells from the immunological and circulatory system) and anti-atherogenic fatty acids (those that prevent plaque aggregation and coronary

Abbreviations: AI, atherogenicity index; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FAME, fatty acid methyl esters; FLQ, flesh lipid quality; GC–MS, gas chromatography–mass spectrometry; HH, hypo- to hyper-cholesterolemic index; HPI, health-promoting index; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; TI, thrombogenicity index; UFA, unsaturated fatty acids; UI, unsaturation index.

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diseases) (Chen & Liu, 2020); the thrombogenicity index (TI) to establish the relationship between pro-thrombogenic and anti-thrombogenic saturated and unsaturated fatty acids, respectively (Chen & Liu, 2020); the hypo-cholesterolemic to hyper-cholesterolemic index (HH) to evaluate the ratio between unsaturated and saturated fatty acids (Chen & Liu, 2020); the health-promoting index (HPI) to evaluate the effect of the composition of fatty acids on cardiovascular health and cardiovascular diseases (Chen & Liu, 2020); the flesh lipid quality (FLQ) to have an indication of the general dietetic quality of lipids and their potential effects on the development of coronary diseases (Chen & Liu, 2020); the n-3 PUFA to n-6 PUFA ratio (n-3/n-6) to assess the proportions of pro-inflammatory and anti-inflammatory lipid mediators in the diet (Chen & Liu, 2020).

The fatty acid composition and the nutritional indices for assessing seafood quality are affected by several factors, among them temperature (Anacleto et al., 2014; Barbosa et al., 2017) and season (Dal Bosco, Mugnai, Mourvaki & Castellini, 2012; Ferreira et al., 2020). The Antarctic community has indicated that climate change will have a direct impact on the diversity and food resources of its marine system (Chown et al., 2012; Turner et al., 2013). A gradual increase in water temperature from global warming may result in changes in species composition (Cheung, Lam, & Pauly, 2008; O'Connor et al., 2007). However, the current understanding on how organisms and ecosystems will respond to atmospheric warming is poor. Hence, the forecasting of individual and population-level responses to environmental changes are among the highest priority questions for the Southern Polar Regions that researchers should address in the next two decades and beyond (Johnston et al., 2019; Kennicutt et al., 2015).

In general, studies on fatty acid composition and nutritional qualities of seafood in response to an increase in temperature are surprisingly limited. The effect of raising the sweater temperature from 18 °C to 22 °C on seabass (*Dicentrarchus labrax*) revealed a considerable 44% decrease in HH and a remarkable 79% increase in n-3/n-6 (Barbosa et al., 2017). Other studies on two bivalve mollusks (*Ruditapes decuss* and *Ruditapes philippinarum*) have observed that the PUSA/SFA, AI, TI, HH and n-3/n-6 are more susceptible to change in the former than the latter species when the temperature was increased from 22 °C to 38 °C (Anacleto et al., 2014).

Fishing activities in the Antarctic region are relatively low in comparison with other parts of the world. However, pressure to exploit Antarctic fisheries is expected to increase as the global population grows. In 1980, the Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR) was created to protect and monitor the exploitation of the Southern Ocean and to ensure the sustainable use of the Southern Ocean (Discovering Antarctica, 2016; Mintenbeck et al., 2012). The Norwegian Ministry of Foreign Affairs has indicated that data from the Antarctic region have gradually been collected, however, the knowledge of the status of fish stocks is limited (Norwegian Ministry of Foreign Affairs, 2016). The previous observations highlight the importance of evaluating nutritional qualities of Antarctic fish species, especially those utilized for human consumption.

Trematomus bernacchii (Emerald Notothen or Emerald Rockcod) is a species of bony fish in the family cod icefishes that has been commercially fished in the Southern ocean for human consumption (Discovering Antarctica, 2016; Kock, Reid, Croxall, & Nicol, 2007), and although the fatty acid profiles in various of its organs have been determined (Antonucci, Belghit, Truzzi, Illuminati, & Araujo, 2019; Gonzalez-Cabrera et al., 1995; Truzzi, Illuminati, Antonucci, Scarponi, & Annibaldi, 2018), there is no information on its nutritional value or the impact of climate change on its nutritional quality indices. While fish from the temperate and tropical latitudes experience much greater seasonal variation in temperature and are correspondingly more thermally tolerant (Aronson, Thatje, McClintock, & Hughes, 2011), Antarctic fish experience negligible seasonal variations from approximately −1.9 °C to 1.8 °C (3.7 °C difference), resulting in a limited ability to adapt to temperature variations and increased vulnerability to climate change effects (Aronson, Thatje, McClintock, & Hughes, 2011).

It is startling the current lack of information about the impact of seawater warming on the nutritional quality indices of fish species of potential interest for human consumption, even though the interest for Antarctic fish stocks date from the turn of the XX century (Miller, 1991). The main goals of the present study are to determine the fatty acid-related nutritional quality indices (PUFA/SFA, AI, TI, HH, HPI, UI, FLQ and n-3/n-6) in muscle of *T. bernacchii* at its natural habitat (-1.87 °C) and at elevated temperatures (0.0, 1.0 and 2.0 °C), to evaluate the effect of seawater warming (0.0, 1.0 and 2.0 °C) on these quality indices. This is the first study reporting the nutritional quality indices of *T. bernacchii* at and above its natural habitat temperature, and consequently it is an important contribution to understand the response of marine organisms of nutritional value to the atmospheric warming in the Antarctic region.

2. Materials and methods

2.1. Chemicals

Methanol and *n*-heptane (Baker, Philipsburg, NJ, USA), acetone and petroleum ether (Carlo Erba, Milano, Italy) were HPLC grade. Sodium methoxide for synthesis (≥97%) was from Merck (Hohenbrunn, Germany). Nonadecanoic acid methyl ester (99.6%) was from Dr. Ehrenstorfer (GmbH, Augsburg, Germany). The 37-component standard mixture of fatty acid methyl esters (FAME) (≥99%) was from Supelco (Bellefonte, PA, USA). Extra pure sodium hydrogen sulfate anhydrous was from Scharlau (Sentmenat, Spain). Helium gas (6.0) was from SOL Group (Monza, Italy).

2.2. Sample collection

The sampling procedure has been described elsewhere (Tuzzi, Illuminati, Antonucci, Scarponi, & Annibaldi, 2018). Briefly, 63 sexually mature specimens of *T. bernacchii* (weight 136–333 g, length 22–30 cm) were caught with a fishing rod in the Ross Sea at the depth of ~ 30 m and seawater temperature of −1.87 °C. Three fish, designated as control seawater (C_{sea}), were immediately sacrificed by a sharp blow to the head, dissected and frozen in liquid N_2 and stored at −80 °C to avoid oxidation.

The remaining fish were acclimatized for 30 days into a seawater tank (1000 L) with a constant flow-through of filtered seawater at −1.8 ± 0.1 °C, a natural photoperiod (24 h daylight), and then transferred randomly into three closed circuit fish tanks at 0.0 ± 0.1, 1.0 ± 0.1 and 2.0 ± 0.1 °C for another 10 extra days. Immediately after the 10 days period, six fish were killed from every tank by a sharp blow on the head, dissected, frozen in liquid N_2 and stored at −80 °C until the analysis. The same diet, consisting of chopped cuttlefish (*Sepia officinalis*) and bivalve molluscs (*Adamussium colbecki*) was provided ad libitum once every second day to all fish during the trials. The experimental temperatures (0.0, +1.0, and + 2.0 °C) were selected considering the predicted shelf water warming of + 0.8 to + 1.48 °C by the year 2200 for the Ross Sea region (Timmermann & Hellmer 2013).

2.3. Lipid extraction

Muscle tissue samples (1 g) from *T. bernacchii* were submitted to microwave assisted extraction (MAE) as described elsewhere (Truzzi, Illuminati, Annibaldi, Antonucci, & Scarponi, 2017). Briefly, the operational parameters were as follows: magnetron power 100%; time to reach settings 10 min; extraction duration 20 min; extraction temperature 90 °C and maximum vessel pressure cut off 1.38×10^6 Pa. After cooling, the extract was filtered through Whatman GF/C filter paper (Ø 90 mm, GE Healthcare Life Sciences, Buckinghamshire, England) filled with sodium hydrogen sulfate anhydrous. The filtrate was evaporated under laminar flow inert gas (N_2) until constant weight (±0.2 mg) and the mass of the extracted lipids was determined by weighting the sample

before and after a freeze-dried process at −20 °C (Edwards EF4, Crawley, Sussex, England). The C_{sea} (-1.87 °C) and the experimental temperatures (0, 1, 2 °C) samples were analyzed in triplicate and sextuplicate, respectively.

2.4. FAME preparation and analysis

The FAME were prepared according to a modified method published elsewhere (ISO 12966–2, 2011) . Briefly, the lipid extract (2.0 to 8.6 mg) was dissolved into 0.5 mL of *n*-heptane and 10 mL of sodium methylate in methanol (2 N), at room temperature, vortex-mixed for 3 min, centrifuged at 67-G for 1 min. The resulting solution was neutralized with 40 mg of sodium hydrogen sulfate anhydrous, mixed for 3 min, and centrifuged at 67-G for 1 min. After the salt settled, 100 μL of the upper phase were transferred to a 1 mL vial, diluted with 400 μL of *n*-heptane and submitted to gas chromatography mass spectrometry analysis (GC–MS).

2.5. Gas chromatography mass spectrometry

An Agilent-6890 GC system coupled to an Agilent-5973 N quadrupole mass selective detector (MSD) was used (Agilent Technologies, Santa Clara, CA, USA). The methylated samples were analyzed on a CC-wax-MS (30 m × 0.25 mm ID, 0.25 μm film thickness) capillary column (CPS Analytica, Milan, Italy). The instrumental parameters were calibrated using the 37-Component FAME mix. The injection volume was 1 μL with a split mode ratio 1:10 using an Agilent glass cup liner, splitless, double taper 5583–4705 (CPS Analytica, Milan, Italy). The inlet temperature was kept at 250 °C. The analysis time was 43 min with helium as carrier gas (5.52×10^5 Pa) and the oven temperature program was as follows: hold time of 1 min at 100 °C, increase from 100 °C to 150 °C at 25 °C/min, from 150 °C to 200 °C at 5 °C/min, and from 200 to 230 °C at 1 °C/min. The ion source, transfer line and detector temperatures were 230, 250 and 150 °C, respectively. The mass spectra were recorded between *m/z* 50 and *m/z* 400 at a rate of three scans per second, with ionization energy of 1.12×10^{-17}J. Data were collected under SIM mode. After a solvent delay of 2.0 min, the following fragment ions were recorded: *m/z* 74 and 87 for saturated, *m/z* 74 and 55 for monoenoic fatty acids, *m/z* 67 and 81 for dienoic fatty acids, and *m/z* 79 and 81 for PUFA. Identification of fatty acids was performed using NIST reference mass spectra database (NIST, Mass Spectral Database 02, National Institute of Standards and Technology, Gaithersburg, MD) MS search 2.0a (NIST 02.L, Ringoes, NJ). The retention time and mass spectra of standard FAME were used to confirm the NIST identification of the fatty acids in the samples.

2.6. Lipids nutritional quality index

Eight nutritional quality indices (PUFA/SFA, AI, TI, HH, HPI, UI, FLQ, n-3/n-6) were calculated in muscle of *T. bernacchii* by means of the formulae provided in Table 1.

2.7. Statistics

Dunnett's test (Dunnett, 1964) was used for comparing the fatty acid (FA) profiles or the quality indices (QI) of the sea control (natural habitat at −1.87 °C) against those recorded at different experimental temperatures (0.0, 1.0 and 2.0 °C). The statistical analysis was carried out by using an automatic Excel calculation platform that is provided as supplementary material (Excel file S1). This calculation file consists of four worksheets: 1) "Data entry": where the data is arranged up to a maximum of 15 conditions (e.g. temperatures) in sextuplicate ($n \leq 6$) and a maximum of 35 variables (e.g. FA or QI) to render a 90 × 35 data matrix. The associated degrees of freedom are displayed automatically in this worksheet. 2) "Normality": where the normality plots of the variables (e.g. FA or QI) are displayed, and their lack of linearity indicates whether the proposed Dunnett parametric test is appropriate for the data under consideration; 3) "Variability Δ%": where the changes between the controls and the experimental conditions are expressed in percentage of variation (Δ%) by using a heat map. The user select a specific percentage of variability (e.g. 30%) to compare the results and the heat map indicates stable in yellow (e.g. − 30%<Δ<+30%), decrease in red (e.g. − 30%>Δ) and increase in green (e.g. Δ>+30%); 4) "Significant Δ%": where the statistical differences between the control and the experimental temperatures (for instance, +2.0 °C) are computed automatically and the significant differences ($p < 0.05$) are indicate with an asterisk (*). The "Original data" discussed in this research (FA and QI at different temperatures and the computed QI from the literature) are included as an extra worksheet in Excel file S1, and can be evaluated by copy-pasting the data into the "Data entry" worksheet. The matrix sizes in the present research for FA (25) and QI (8) were 21 × 25 and 21 × 8, respectively. The number of rows ($21 = 1 \times 3 + 3 \times 6$) corresponds with the sea control ($n = 3$) and the three experimental temperatures ($n = 6$ for each temperature).

3. Results

3.1. Fatty acids

The extracted muscle lipid fractions from *T. bernacchii* at environmental (-1.87 °C) and experimental (0.0, 1.0, 2.0 °C) temperatures were submitted to transesterification followed by fatty acid analysis by

Table 1
List of nutritional quality index and their mathematical expression.

Quality index	Mathematical expression
Polyunsaturated to saturated fatty acid ratio	$\mathrm{PUFA/SFA} = \frac{\sum \mathrm{PUFA}}{\sum \mathrm{SFA}}$
Atherogenicity index	$\mathrm{AI} = \frac{[12:0 + (14:0 \times 4) + 16:0]}{\sum \mathrm{UFA}}$
Thrombogenicity index	$\mathrm{TI} = \frac{[14:0 + 16:0 + 18:0]}{\left[(\sum \mathrm{MUFA} \times 0.5) + (\sum \mathrm{PUFAn-6} \times 0.5) + (\sum \mathrm{PUFAn-3} \times 3) + \left(\frac{\sum \mathrm{n-3}}{\sum \mathrm{n-6}}\right)\right]}$
Hypo- to hyper-cholesterolemic ratio	$\mathrm{HH} = \frac{(\mathrm{cis-18:1} + \sum \mathrm{PUFA})}{(12:0 + 14:0 + 16:0)}$
Health-promoting index	$\mathrm{HPI} = \frac{\sum \mathrm{UFA}}{[12:0 + (14:0 \times 4) + 16:0]}$
Unsaturation index	$\mathrm{UI} = 1 \times (\%\mathrm{monoenoics}) + 2 \times (\%\mathrm{dienoics}) + 3 \times (\%\mathrm{trienoics}) + 4 \times (\%\mathrm{tetraenoics}) + 5 \times (\%\mathrm{pentaenoics}) 6 \times (\%\mathrm{hexaenoics})$
Flesh lipid quality	$\mathrm{FLQ} = 100 \times \left(\frac{22:6n-3 + 20:5n-3}{\sum \mathrm{FA}}\right)$
Omega-3/omega-6 ratio	$\mathrm{n-3/n-6} = \frac{\sum (n-3)\mathrm{PUFA}}{\sum (n-6)\mathrm{PUFA}}$

Polyunsaturated to saturated fatty acids ratio (PUFA/SFA), Atherogenicity index (AI), Thrombogenicity index (TI), Hypo- to hyper-cholesterolemic index (HH), Health-promoting index (HPI), Unsaturation index (UI), Flesh lipid quality (FLQ) were calculated according to Chen & Liu (2020).
Omega-3/omega-6 ratio (n-3/n-6) was calculated according to Ramos Filho, Ramos, Hiane, & De Souza (2010).

GC–MS. The concentration profiles in relative units (%) of the 25 detected FAME (saturated, mono-unsaturated and poly-unsaturated) at the different experimental conditions are presented in Table 2. In general, the comparison of the sea control (-1.87 °C) with the experimental temperatures (Table 3) revealed significant increase in the levels of SFA and PUFA and significant decrease in the levels of MUFA and UFA at 0.0 and 1.0 °C, respectively. In contrast, the changes for these parameters (SFA, PUFA, MUFA, UFA) were not statistically significant at 2 °C.

The comparison Δ% values in Table 3 revealed that the SFA exhibited larger Δ% values at 0 °C (71%) or 1 °C (100%) than at 2 °C (29%). In addition, Table 3 showed that the number of fatty acids with the largest absolute Δ% increases from 4 (16:1n-7, 18:1n-7, 20:2n-6 and 18:3n-6, at 0 °C) to 13 (12:0, 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 17:1n-7, 18:1n-9, 18:2n-6, 18:3n-3, 20:4n-6 and 20:5n-3, at 1 °C) and declines to 8 (14:1n-5, 20:1n-9, 22:1n-9, 24:1n-9, 16:2n-7, 20:3n-6, 20:3n-3 and 22:6n-3, at 2 °C).

3.2. Quality indices

Eight quality indices were determined by using the formulae in Table 1 and their inverse normal plots showed a high degree of linearity (Fig. S1). The calculated quality indices (Table 4) and their statistical analysis (Table 5) revealed significant increases in UI, FLQ and n-3/n-6 and significant decrease in HH at 0 and 1 °C, respectively. The quality indices PUFA/SFA and HPI were significantly increased at 0 and 2 °C, respectively, while AI was significantly decreased at 0 °C and 2 °C. TI was characterized by a non-significant variability at every temperature.

Table 2
Percentage of total lipids (bracketed values) and total fatty acids in muscle of *Trematomus bernacchii* caught in the Ross Sea (C_{sea} = -1.87 °C) and after 10 days exposure to 0, +1 and + 2 °C. Values for C_{sea} (n = 3) and 10 Days (n = 6) are expressed as average and standard deviation ($\mu \pm \sigma$). Dunnett's test was used for comparing 10 Days against C_{sea} ($p < 0.05$) and the significant values are indicated by an asterisk.

Fatty acids	C_{sea}(5.2 ± 1)	10 Days 0 °C(3.4 ± 0.5) *	+1°C(2.5 ± 0.5) *	+2°C(2.0 ± 0.5) *
12:0	0.12 ± 0.01	0.16 ± 0.02*	0.18 ± 0.01*	0.14 ± 0.01
14:0	8.38 ± 0.14	6.96 ± 0.19*	6.71 ± 0.19*	6.90 ± 0.23*
15:0	0.29 ± 0.01	0.40 ± 0.02*	0.45 ± 0.02*	0.36 ± 0.03*
16:0	9.10 ± 0.08	11.3 ± 0.3*	12.1 ± 0.2*	10.2 ± 0.4*
17:0	0.10 ± 0.01	0.21 ± 0.01*	0.25 ± 0.01*	0.13 ± 0.01*
18:0	1.36 ± 0.01	2.59 ± 0.07*	2.63 ± 0.09*	1.65 ± 0.02*
20:0	0.05 ± 0.01	0.10 ± 0.01*	0.10 ± 0.01*	0.10 ± 0.01*
Total SFA	*19.4 ± 0.2*	*21.7 ± 0.5**	*22.4 ± 0.3**	*19.5 ± 0.6*
14:1n-5	0.40 ± 0.01	0.39 ± 0.01	0.45 ± 0.01*	0.34 ± 0.02*
16:1n-7	11.5 ± 0.1	10.2 ± 0.4*	11.7 ± 0.6	12.3 ± 0.2*
17:1n-7	0.43 ± 0.01	0.56 ± 0.04*	0.64 ± 0.07*	0.57 ± 0.03*
18:1n-9	26.8 ± 0.1	20.0 ± 0.7*	19.8 ± 0.8*	27.4 ± 0.3
18:1n-7	6.52 ± 0.03	5.48 ± 0.36*	5.80 ± 0.09*	6.06 ± 0.06*
20:1n-9	5.59 ± 0.03	5.54 ± 0.55	5.67 ± 0.41	4.89 ± 0.14*
22:1n-9	2.19 ± 0.04	2.31 ± 0.12	2.29 ± 0.14	2.01 ± 0.11
24:1n-9	0.99 ± 0.02	0.97 ± 0.05	0.85 ± 0.04*	0.80 ± 0.04*
Total MUFA	*54.4 ± 0.1*	*45.5 ± 0.7**	*47.1 ± 0.5**	*54.3 ± 0.3*
16:2n-7	0.08 ± 0.01	0.07 ± 0.01	0.07 ± 0.01*	0.05 ± 0.01*
18:2n-6	2.45 ± 0.02	2.16 ± 0.04*	2.03 ± 0.02*	2.10 ± 0.04*
20:2n-6	0.31 ± 0.01	0.50 ± 0.02*	0.50 ± 0.04*	0.45 ± 0.02*
18:3n-6	0.19 ± 0.01	0.29 ± 0.03*	0.28 ± 0.03*	0.27 ± 0.02*
18:3n-3	0.83 ± 0.02	0.85 ± 0.04	0.70 ± 0.05*	0.71 ± 0.04*
20:3n-6	0.27 ± 0.02	0.18 ± 0.01*	0.16 ± 0.01*	0.15 ± 0.01*
20:3n-3	0.09 ± 0.01	0.16 ± 0.01*	0.14 ± 0.01*	0.16 ± 0.01*
20:4n-6	0.58 ± 0.02	0.86 ± 0.04*	0.99 ± 0.10*	0.81 ± 0.03*
20:5n-3	10.3 ± 0.1	15.2 ± 0.5*	15.4 ± 0.4*	13.1 ± 0.2*
22:6n-3	10.9 ± 0.2	12.4 ± 0.8*	10.0 ± 0.5	8.25 ± 0.36*
Total PUFA	*26.0 ± 0.3*	*32.6 ± 1.1**	*30.3 ± 0.5**	*26.0 ± 0.4*
Total UFA	*80.4 ± 0.2*	*78.1 ± 0.5**	*77.5 ± 0.3**	*80.3 ± 0.6*

SFA: Saturated Fatty Acids; MUFA: Mono Unsaturated Fatty Acids; PUFA: Poly Unsaturated Fatty Acids; UFA: Unsaturated Fatty Acids.

Table 3
Comparison of the fatty acid profiles in muscle of *T. bernacchii* for seawater control (-1.87 °C) against those recorded at different experimental temperatures (0.0, +1.0 and + 2.0 °C) and expressed as percentage of variation (Δ%). Statistically significant differences were determined by a Dunnett test and indicated with an asterisk ($p < 0.05$).

Fatty acid	Δ% 0 °C	+1°C	+2°C
12:0	+39*	+55*	+17
14:0	−17*	−20*	−18*
15:0	+35*	+53*	+24*
16:0	+24*	+32*	+12*
17:0	+116*	+161*	+37*
18:0	+91*	+94*	+22*
20:0	+81*	+88*	+82*
Total SFA	+12*	+15*	0
14:1n-5	−4	+12*	−16*
16:1n-7	−11*	+1	+6*
17:1n-7	+29*	+48*	+32*
18:1n-9	−25*	−26*	+2
18:1n-7	−16*	−11*	−7*
20:1n-9	−1	+2	−12*
22:1n-9	+5	+4	−8
24:1n-9	−2	−14*	−19*
Total MUFA	−16*	−13*	0
16:2n-7	−11	−18*	−40*
18:2n-6	−12*	−17*	−14*
20:2n-6	+61*	+58*	+44*
18:3n-6	+57*	+51*	+47*
18:3n-3	+2	−16*	−15*
20:3n-6	−32*	−39*	−42*
20:3n-3	+71*	+57*	+72*
20:4n-6	+48*	+71*	+39*
20:5n-3	+48*	+50*	+27*
22:6n-3	+13*	−8	−24*
Total PUFA	+26*	+17*	+0
Total UFA	−3*	−4*	+0

Table 4
Values of the quality indexes in the muscle of Antarctic fish *Trematomus bernacchii* of the caught fish in the Ross Sea (C_{sea}) and after 10 days exposure to 0.0, +1.0 and + 2.0 °C. The values at C_{sea} and 10 Days are expressed as average and standard deviation ($\mu \pm \sigma$) for n = 3 and n = 6, respectively.

Nutritional Index	C_{sea}	10 Days 0 °C	+1°C	+2°C
PUFA/SFA	1.32 ± 0.03	1.50 ± 0.08	1.35 ± 0.04	1.33 ± 0.06
AI	0.53 ± 0.01	0.50 ± 0.02	0.50 ± 0.01	0.47 ± 0.02
TI	0.19 ± 0.01	0.18 ± 0.01	0.19 ± 0.01	0.18 ± 0.01
HH	3.37 ± 0.06	3.16 ± 0.10	2.95 ± 0.08	3.46 ± 0.16
HPI	1.88 ± 0.03	1.99 ± 0.07	1.98 ± 0.05	2.12 ± 0.09
UI	183 ± 1	209 ± 6	197 ± 3	181 ± 2
FLQ	21.2 ± 0.2	27.6 ± 1.2	25.5 ± 0.6	21.3 ± 0.3
n-3/n-6	5.81 ± 0.02	7.14 ± 0.38	6.64 ± 0.35	5.85 ± 0.09

Table 5
Comparison of the nutritional quality indices in muscle of *T. bernacchii* for the seawater control (-1.87 °C) against those recorded at different experimental temperatures (0.0, +1.0 and + 2.0 °C) and expressed as percentage of variation (Δ%). Statistically significant differences were determined by a Dunnett test and indicated with an asterisk ($p < 0.05$).

FAs	Δ% 0 °C	+1°C	+2°C
PUFA/SFA	+14*	+2	+1
AI	−6*	−5	−11*
TI	−5	+3	−1
HH	−6*	−12*	+3
HPI	+6	+6	+13*
UI	+14*	+8*	−1
FLQ	+30*	+20*	+1
n-3/n-6	+23*	+15*	+1

Four computed indices (PUFA/SA, HPI, FLQ and n-3/n-6) increased and only AI decreased at every tested temperature (0.0, 1.0, 2.0 °C) compared to the sea control at −1.87 °C.

4. Discussion

It is undeniable the impact of climate change on the metabolism of living organisms (Brodersen et al., 2011; Paital & Chainy, 2014). Changes in temperature have been associated with alterations in tissue lipid composition, cellular membrane fluidity (Constable et al., 2014; Hixson & Arts, 2016) and gene expression of fish exposed at different environmental temperatures (Windisch et al., 2014). The tolerance of *T. bernacchii* (natural habitat −1.87 °C) to high or low temperatures is remarkably narrow. Its lower boundary or temperature at which it freezes is − 2.2 °C and its upper incipient lethal temperature, a parameter currently used to measure the temperature at which median mortality is no longer time dependent, has been established between 5 and 7 °C (Bilyk, 2011). Antarctic fish retain the capacity to compensate for chronic temperature change by displaying astounding plasticity in metabolic control (Seebacher, Davison, Lowe, & Franklin, 2005). This metabolic control can be used to explain the consistent lack of statistical significance changes for SFA, PUFA, MUFA and UFA at + 2.0 °C. However, some authors have mentioned that the efficiency of the compensation process is negatively affected at elevated temperatures (Windisch et al., 2014; Feidantsis et al., 2020). It is likely that the combined dynamic of the two processes (compensation and negative temperature effect) explains the observed larger values of Δ% for SFA at 0 and 1 °C (71 and 100%) as compared to 2 °C (29%), and the increase in the number of fatty acids that experienced the largest change in relative concentration (Δ%) at 0, 1 and 2 °C from 4 to 13 and further decrease to 8, respectively.

The observed decrease in concentration for 16:1n-7, 18:1n-9 and increase for 22:1n-9, 18:3n-3 and 22:6n-3 at 0 °C compared with the seawater control group (-1.87 °C) are consistent with Gonzalez-Cabrera et al. (1995). These authors also observed the same trends for these fatty acids in muscle of *T. bernacchii* in a warm-acclimation study. It is possible that the significant decrease of DHA at 2 °C is due to its precursor role in the production of active biological molecules that are promoted significantly by the action of the increased temperature. However, some researchers have suggested that several other factors are likely to affect the variability of DHA under warmer temperature in stenothermal fish species (Brodte, Graeve, Jacob, Knust, & Pörtner, 2008).

The nutritional value of dietary food is generally assessed by means of nutritional indices. However, there are not recommendations about the intake of fish with specific nutritional quality indices. Estimated nutritional index ranges from different fish species (e.g., salmon, trout, carp, tilapia, corvina, seabass, pompano, puffer, etc) have varied between 0.50 and 1.62 for PUFA/SFA, 0.21–1.41 for AI, 0.14–0.87 for TI, 1.54–4.83 for HH, 13.01–36.37 for FLQ, 2.9–12.4 for n-3/n-6 (Chen & Liu, 2020). The corresponding nutritional indices (Table 4) for *T. bernacchii*, in its natural habitat (-1.87 °C) and at the different experimental temperatures (0.0, +1.0 and + 2.0 °C) are within the previously mentioned ranges for different fish species (Chen & Liu, 2020).

There is a lack of research on the impact of seawater warming on the nutritional quality indices of Antarctic fish. The reported means and standard deviations for fatty acid levels in muscle of *T. bernacchii* in a study concerned with warm-acclimation at −1.5 and 4 °C (Gonzalez-Cabrera et al., 1995) were used to estimate some nutritional indices at −1.5 °C (Excel file S1). The estimated values for PUFA/SFA (1.68 ± 0.47), TI (0.17 ± 0.03), HH (2.48 ± 0.53) and UI (267 ± 130) do not differ from the values calculated in the present research (1.32 ± 0.03, 0.19 ± 0.01, 3.37 ± 0.06 and 183 ± 1, respectively) at a 95% confidence level. Although, there was some statistically significant difference between the estimated AI (0.40 ± 0.05) from Gonzalez-Cabrera et al. (1995) and the present work (0.53 ± 0.01) at $p < 0.05$ (but not at $p < 0.03$), the difference in question may indicate some biological variation. This biological variation is reflected in the acceptable percentage of variability between the two values ($\Delta < 25\%$) and the lack of significance at the 97% confidence level.

The computed PUFA/SFA, AI, TI, HPI and FLQ in muscle of *T. bernacchii* in the present work resemble the behavior of these quality indices in warm-acclimation studies of other non-Antarctic fish species published elsewhere (Anacleto et al., 2014; Barbosa et al., 2017; Dal Bosco, Mugnai, Mourvaki & Castellini, 2012; Ferreira et al., 2020).

The involvement of dietary fish in different pathologies and their potential use for disease prevention and treatment are aspects that enhance the importance of defining nutritional quality indices to understand the quality of lipids in fish (Chen & Liu, 2020; Ramos Filho, Ramos, Hiane, & De Souza, 2010). For instance, PUFA/SFA, AI, TI, HH and n-3/n-6 have been used to assess the impact of diet on cardiovascular health (Chen & Liu, 2020; Ramos Filho, Ramos, Hiane, & De Souza, 2010; Ivanova & Hadzhinikolova, 2015; Pleadin et al., 2017). The HPI, UI and FLQ have been used as standards for evaluating the content of high-quality PUFA (Chen & Liu, 2020). It has been reported that PUFA/SFA under 0.45 promotes hypercholesterolemia (Ivanova & Hadzhinikolova, 2015; Ramos Filho, Ramos, Hiane, & De Souza, 2010), consequently a ratio PUFA/SFA around 1.0 ± 0.2 is regarded as an optimum dietary value (Chen & Liu, 2020; Ivanova & Hadzhinikolova, 2015). Positive effecs on human health have been ascribed to TI values lower than 1 and HH values around 2.40 (Ivanova & Hadzhinikolova, 2015; Pleadin et al., 2017). Based on these healthy reported values, the estimated PUFA/SFA (1.32 ± 0.03), AI (0.53 ± 0.01), TI (0.19 ± 0.01) and HH (3.37 ± 0.06) from muscle of *T. bernacchii* can be regarded as optimal indices that give further credence to the nutritional potential of *T. bernacchii*. A comparison of the nutritional quality indices for *T. bernacchii* in its natural habitat (-1.87 °C) and other fish species of commercial value (Table S1) indicates that the indices for *T. bernacchii* are comparable (and in some instances superior) to other fish species of commercial and nutritional value for humans.

5. Conclusion

The fatty acid quality indices in muscle of *T bernacchii*, namely PUFA/SFA, AI, TI, HH, FLQ and n-3/n-6, were within the expected ranges for dietary fish.

The present research indicated that a gradual increase in water temperature from global warming may result in acclimation to higher heat tolerance. Warming of the sea water between −1.87 and 2 °C caused changes in the fatty acid composition and the nutritional quality indices from muscle of *T. bernacchii* in the range of $-12\% < \Delta < 30\%$. It was confirmed, by using published data, that the observed changes in muscle of *T. bernacchii* were not statistically significant and attributable to biological variability. Consequently, the nutritional value of muscle of *T. bernacchii* is preserved after increasing the temperature of its normal habitat by ~ 4°. The indices PUFA/SFA, AI, TI, HPI FLQ and n-3/n-6 in muscle of *T bernacchii* resembled the behavior of non-Antarctic fish in warm-acclimation studies.

CRediT authorship contribution statement

Pedro Araujo: Conceptualization, Data curation, Formal analysis, Investigation, Project administration, Resources, Software, Supervision, Visualization, Writing - original draft, Writing - review & editing. **Cristina Truzzi:** Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing - original draft, Writing - review & editing. **Ikram Belghit:** Conceptualization, Data curation, Formal analysis, Investigation, Supervision, Visualization, Writing - original draft, Writing - review & editing. **Matteo Antonucci:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision,

Validation, Visualization, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2021.130500.

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