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ORIGINAL PAPER

Modeling the influence of time and temperature on the levels of fatty acids in the liver of Antarctic fish *Trematomus bernacchii*

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Abstract

Antarctic fish (*Trematomus bernacchii*) are an ideal group for studying the effect of ocean warming on vital physiological and biochemical mechanisms of adaptation, including changes in the fatty acid composition to higher heat tolerance in the sub-zero waters of the Southern Ocean. Despite the awareness of the impact of ocean warming on marine life, bioclimatic models describing the effect of temperature and time on fatty acid levels in marine species have not been considered yet. The objective of the present study was to investigate changes in the concentrations of fatty acids in liver from *T. bernacchii* in response to an increase in temperature in the Antarctic region. Changes in the concentrations of fatty acids in liver from *T. bernacchii* were observed after varying simultaneously and systematically the temperature and time. The fatty acid profiles were determined by gas chromatography prior to acclimation (− 1.8 °C) and after acclimation (0.0, 1.0, and 2.0 °C) at different times (1, 5, and 10 days). The observed changes were graphically visualized by expressing the fatty acid concentration in absolute units (mg g^{-1}) as a function of the temperature and time using polynomial models. Major changes in fatty acid composition were observed at day 1 of exposition at all temperatures. At day 5, the fish seem to tolerate the new temperature condition. The concentrations of saturated fatty acids were almost constant throughout the various conditions. The concentrations of monounsaturated fatty acids (in particular 18:1$n-9$) decrease at day 1 for all temperatures. In contrast, there was an increase in the concentrations of polyunsaturated fatty acids (in particular 20:5$n-3$ and 22:6$n-3$) with increasing temperatures after 1, 5, and 10 days of exposure. The proposed models were in agreement with reported studies on polar and temperate fish, indicating possibly similar adaptation mechanisms for teleost to cope with global warming.

Keywords Global warming · Bioclimatic models · Thermal acclimation · Gas chromatography · Nototheniidae species · Lipid composition

Introduction

Local climate models are representations of the likely effect of anthropogenic sources on climate (Intergovernmental Panel for Global Climate Change (IPCC) 2000). Bioclimatic models have predicted the extinction of Antarctic toothfish in 30 years under strong warming conditions (Cheung et al. 2008). Data from Pleistocene glaciation have indicated that species more often responded to climate change by local adaptation (Parmesan et al. 2000). It has been reported that gradual increase in water temperature from global warming may result in changes in species composition and acclimation to higher heat tolerance (O'Connor et al. 2007; Cheung et al. 2008). It is undeniable that sea temperature is the principal factor determining distributions, abundance, and physiology of fish species (Pörtner 2001; Roessig et al. 2004). The Commission for the Conservation of the Antarctic Marine Living Resources has pointed out that the Antarctic region and more specifically the Ross Sea have not been dramatically affected by commercial fisheries, whaling, and other human activities when compared to its north counterpart (Antarctic and Southern Ocean Coalition (ASOC) 2009). For this particular reason, this protected region without

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many human population or other anthropomorphic interference provides a unique potential for documenting the biological and chemical effects of global warming on a polar marine ecosystem (Antarctic and Southern Ocean Coalition (ASOC) 2009). Fatty acids (FA) are regarded as ideal biological markers of environmental exposure in marine research, because they are able to objectively assess dietary intake/status and to identify key processes impacting the dynamics of some of the major ecosystems in the world (Dalsgaard et al. 2003; Könneke and Widdel 2003; Alfaro et al. 2006; Sajjadi and Eghtesadi-Araghi 2011; Copeman et al. 2013; Mourente et al. 2015). Recently, the fatty acid levels of *T. bernacchii* have been experimentally determined in liver, muscle, and gills (Truzzi et al. 2017, 2018a, b; Corsolini and Borghesi 2017; Malekar et al. 2018).

There is a paucity in information regarding the behavior of the fatty acid profiles when the factors time and temperature are varied simultaneously. The majority of published studies are mainly concerned with the effect of the temperature on the concentrations of fatty acids without considering temporal changes (Ramesha and Thompson 1982; Dey et al. 1993; Buda et al. 1996; Lahdes et al. 2000; Brodte et al. 2008). Some authors have investigated the temporal effect of temperature and tissue type (liver and muscle) on the levels of fatty acids in fish (Copeman et al. 2013). However, the main drawback of this study is that influence of the temperature and time were evaluated at two and five levels, respectively, which precluded the modeling of the factors, as reflected by the lack of degrees of freedom to judge the adequacy of the temperature (at two levels) in a basic model of the form $y = mx + b$ (only one distinct line can be drawn through two different points).

The hypothesis of the present research is to investigate whether mathematical modeling of selected environmental variables (time and temperature) can predict changes in the fatty acid concentration of Antarctic fish *T. bernacchii*. This ubiquitous species of stenothermal Antarctic teleost (family Nototheniidae) is well adapted to the extremely low temperatures of the Antarctic region (− 1.86 °C) and has been regarded as a key indicator for monitoring anthropogenic impact (Regoli et al. 2005; Di Bello et al. 2007; Illuminati et al. 2010). Considering the important role of lipids in polar fish, the aim of the present study was to investigate the warm acclimation response of *T. bernacchii* exposed to higher temperatures. It is important to mention that the present research covers some of the 80 priority questions for the Southern Polar Regions that were formulated by researchers during the First Scientific Committee on Antarctic Research, more specifically: predictions of the response of the Antarctic to a warming world (Kennicutt et al. 2015). Climate change has raised concerns over the fate of notothenioids. Although after many decades of research on the adaptation of polar fish to climate warming, there are still many questions that remain to be investigated. The present study provides information on the acclimation of the dominant fish sub-order to a thermal shock, which helps to better understand the response of these Antarctic species to the global warming. In addition, the computed models used in the present study may predict changes in the lipid profile of Antarctic *T. bernacchii* in relation to the global warming.

Materials and methods

Reagents

Sodium hydroxide, hexane, methanol, boron trifluoride in methanol (20% w v^{-1}), and chloroform were purchased from Merck (Darmstadt, Germany). Cod liver oil standard was from Peter Møller (Lysaker, Norway), and fatty acid methyl ester (FAME) pure and model mixture standards (Online Resource 1) were purchased from Nu-Chek Prep (Elysian, MN). Nonadecanoic acid methyl ester (19:0) internal standard was from Fluka (Buchs, Switzerland). De-ionized water was purified in a Milli-Q system (Milli-Q system Millipore, Milford, MA).

Sampling

The experimental study with Antarctic *T. bernacchii* is reported in detail elsewhere (Truzzi et al. 2018a, b). Briefly, the study was performed at the Mario Zucchelli Station at the Terra Nova Bay in the Antarctic region (Fig. 1) during the Austral summer 2014 (a particular period that is characterized by the lack of ice in the Ross Sea), by following the institutional and national guidelines for the care and use of animals and approved by the Italian Ministry of Foreign Affairs. The Code of Conduct of the Scientific Committee on Antarctic Research (SCAR 2011) was followed for the handling and sacrificing of animals. A total of 63 sexually mature Antarctic *T. bernacchii* species (weight 113–503 g, length 32–22 cm) were caught by a fishing rod at depths of 30 m in the location indicated in Fig. 1. The seawater temperature at the moment of the capture was − 1.87 °C. Three fish were immediately sacrificed by a sharp blow to the head (designated as control seawater at 0 day and -1.8 ± 0.2 °C in Table 1) and their liver isolated and immediately frozen in liquid N_2 and stored at − 80 °C to preserve them from oxidation and ensure their integrity. Following collection, the rest of the fish (60) were transferred into a net to Mario Zucchelli Station and placed into a flow-through circulating seawater tank containing 1000 L of filtered running seawater at -1.8 ± 0.2 °C for acclimation at habitat temperature for 30 days. The overview of the sampling flow is portrayed in Online Resource 2. The fish were maintained under a natural photoperiod (24 h daylight). After the acclimation period,

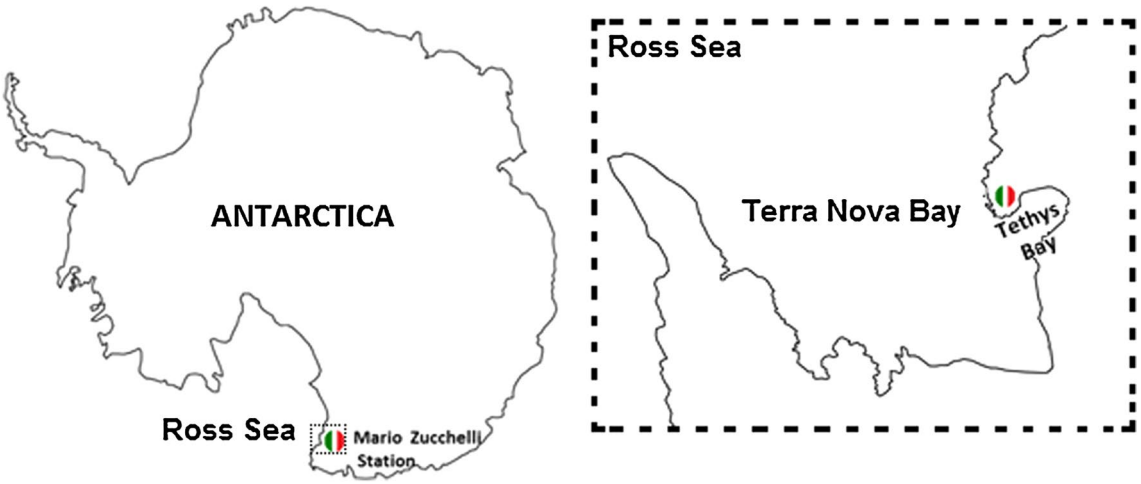

Fig. 1 Location of the sampling point at the Mario Zucchelli Station (74°42′052″ South, 164°02′267″ East), Antarctic region

six fish were killed by a sharp blow on the head, their liver isolated, frozen in liquid N_2, and stored at − 80 °C until analysis (designated as control after acclimation in Table 1). The rest of the acclimatized fish (54) were divided into three different groups and transferred to three new flow-through circulating seawater tanks at pre-established temperatures (0.0, + 1.0, and + 2.0 °C) and held at these conditions for 1, 5, and 10 days (Online Resource 2). The choice of temperatures of 0, + 1, and + 2 °C as thermal stress was based on the shelf water warming of + 0.8 to + 1.48 °C predicted by the year 2200 for the Ross Sea region (Timmermann and Hellmer 2013). Six fish were killed, at each time period, their liver isolated, frozen in liquid N_2, and stored at − 80 °C until analysis. Fish were not starved before each sampling period. The same diet was provided ad libitum once every second day to all fish during the trials (including acclimation period). The diet, sampled at the same period and area, consisted of chopped cuttlefish (*Sepia officinalis*) and bivalve molluscs (*Adamussium colbecki)* and it was kept in the freezer throughout the experimental trial. The same batch of diet was given to the different groups of fish in the same amounts during the acclimation and experimental periods. A comparison of the fatty acid profiles of the caught fish in the Ross Sea (control seawater) and the fish acclimatized for 30 days was performed to check for differences before and after the acclimatization period and establish whether the experimental diet match the natural diet of *T. bernacchii* in terms of fatty acids.

Lipid extraction and fatty acid determination

The sampled livers were pooled together (3 livers for the control seawater, 6 for acclimation, and 6 for every specific time/temperature condition), minced and homogenized by means of a high dispersing device (Ultra-Turrax T25, Janke e Kunkel, IKA-Labortechnik, Staufen, Germany). The pooling of the samples was based on the characterization of biological variations in terms of an effective number, e.g., the average pooled concentration (Gregorius 1991, 2016). The effective number is a familiar notion in ecology, which results from equating an observed dispersion with the dispersion of an ideal community and solving for the number of types in the latter (Gregorius and Gillet 2015). When applied to the present fish community, the concept of concentration effective number requires the definition of an ideal community (corresponding to the control seawater at 0 day and − 1.8 ± 0.2 °C), that is equated to a pooled average value *X*. Consequently, any other community at different day and temperature with the same *X* value is equivalent to the control seawater. It is important to mention that not all dispersion measures based on dissimilarities allow for the computation of concentration effective numbers. For example, nutritional models evaluating the impact of different diets and where the notion of the ideal diet is unknown require computing the biological variability of the individuals within the groups (Gregorius and Gillet 2015). The mathematics behind the requirements for asserting the validity of an effective number are described elsewhere (Gregorius 1991, 2016; Jost 2010; Gregorius and Gillet 2015; Daly 2018). The lipids were extracted in duplicate ($n = 2$) by adding 4 mL of chloroform/methanol (2: 1, v v^{-1}) and 0.080 mL of internal standard (19:0 methyl ester) to 200 mg of sample. The extracted lipids were filtered, the residue was discarded, and the chloroform/methanol filtrate was evaporated under a stream of nitrogen. The dried residue was saponified according to a method published elsewhere (Torstensen et al. 2004). Briefly, an aliquot (1 mL) of sodium hydroxide in methanol (0.5 M) was added, heated at 100 °C for 15 min, and cooled in cold water. An aliquot (2 mL) of boron trifluoride in methanol (20%) was added, vortex-mixed, heated

Table 1 Absolute concentrations of fatty acids (mg g^{-1}) in the liver of Antarctic fish *Trematomus bernacchii* of the caught fish in the Ross Sea (0 day and − 1.87 °C), fish acclimatized for 30 days in a circulating seawater tank control at − 1.87 °C, and at different times (1, 5, 10 days) and temperatures (0.0, 1.0, 2.0 °C) after the acclimation period

Time (days)	0	Acclimatized	1	5	10	1	5	10	1	5	10
Temperature (°C)	− 1.87	− 1.87	0.00	0.00	0.00	1.00	1.00	1.00	2.00	2.00	2.00
14:0	0.96 ± 0.15	0.57 ± 0.04	0.39 ± 0.04	1.03 ± 0.01	0.58 ± 0.07	0.25 ± 0.02	0.51 ± 0.01	0.32 ± 0.09	0.28 ± 0.02	0.85 ± 0.00	0.60 ± 0.04
16:0	5.36 ± 0.39	3.99 ± 0.43	3.82 ± 0.22	5.13 ± 0.16	4.91 ± 0.72	2.75 ± 0.23	4.20 ± 0.13	3.62 ± 0.24	3.30 ± 0.13	4.75 ± 0.03	5.17 ± 0.46
18:0	1.14 ± 0.08	1.00 ± 0.10	0.96 ± 0.07	1.26 ± 0.07	1.18 ± 0.15	0.77 ± 0.03	1.09 ± 0.10	1.06 ± 0.04	0.78 ± 0.03	1.11 ± 0.01	1.24 ± 0.12
18:3$n-3$	0.07 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.08 ± 0.00	0.09 ± 0.02	0.06 ± 0.00	0.10 ± 0.01	0.05 ± 0.03	0.04 ± 0.00	0.09 ± 0.01	0.06 ± 0.00
20:5$n-3$	2.50 ± 0.14	3.20 ± 0.78	4.19 ± 0.16	3.94 ± 0.05	4.51 ± 0.87	2.94 ± 0.14	4.71 ± 0.50	4.76 ± 0.61	3.44 ± 0.10	5.18 ± 0.16	3.19 ± 0.20
22:5$n-3$	0.11 ± 0.01	0.17 ± 0.03	0.25 ± 0.02	0.21 ± 0.00	0.43 ± 0.09	0.12 ± 0.00	0.25 ± 0.01	0.21 ± 0.07	0.15 ± 0.01	0.21 ± 0.00	0.18 ± 0.10
22:6$n-3$	3.31 ± 0.16	5.01 ± 1.43	6.95 ± 0.04	5.98 ± 0.09	7.35 ± 1.55	4.14 ± 0.22	7.39 ± 0.67	7.25 ± 1.31	4.98 ± 0.16	8.60 ± 0.34	4.79 ± 0.36
18:2$n-6$	0.41 ± 0.04	0.32 ± 0.04	0.38 ± 0.04	0.40 ± 0.01	0.40 ± 0.07	0.21 ± 0.02	0.39 ± 0.02	0.22 ± 0.08	0.18 ± 0.01	0.47 ± 0.00	0.33 ± 0.01
20:4$n-6$	0.65 ± 0.04	0.64 ± 0.12	0.94 ± 0.04	0.67 ± 0.01	0.86 ± 0.13	0.47 ± 0.01	0.84 ± 0.06	0.79 ± 0.10	0.68 ± 0.03	0.83 ± 0.02	0.64 ± 0.00
16:1$n-9$	0.36 ± 0.02	0.41 ± 0.05	0.33 ± 0.04	0.37 ± 0.01	0.42 ± 0.07	0.21 ± 0.00	0.43 ± 0.00	0.34 ± 0.05	0.18 ± 0.01	0.44 ± 0.00	0.51 ± 0.06
18:1$n-9$	7.57 ± 0.69	5.92 ± 0.56	4.83 ± 0.60	9.16 ± 0.39	5.87 ± 0.74	2.71 ± 0.20	5.94 ± 0.58	3.57 ± 1.00	2.02 ± 0.13	6.36 ± 0.02	6.79 ± 0.40
20:1$n-9$	0.84 ± 0.05	0.73 ± 0.05	0.69 ± 0.05	0.74 ± 0.01	0.74 ± 0.09	0.35 ± 0.02	0.68 ± 0.02	0.65 ± 0.07	0.42 ± 0.01	0.76 ± 0.00	0.72 ± 0.04
22:1$n-9$	0.18 ± 0.02	0.17 ± 0.02	0.24 ± 0.00	0.24 ± 0.00	0.28 ± 0.05	0.17 ± 0.01	0.22 ± 0.02	0.18 ± 0.07	0.18 ± 0.00	0.28 ± 0.02	0.22 ± 0.03
16:1$n-7$	3.05 ± 0.19	3.12 ± 0.34	2.70 ± 0.29	8.32 ± 0.42	3.88 ± 0.61	1.81 ± 0.12	3.39 ± 0.13	2.23 ± 0.71	1.04 ± 0.05	2.85 ± 0.04	4.11 ± 0.52
18:1$n-7$	2.90 ± 0.27	2.33 ± 0.22	2.07 ± 0.16	2.69 ± 0.06	2.65 ± 0.42	1.39 ± 0.08	2.53 ± 0.12	1.88 ± 0.24	1.43 ± 0.08	2.46 ± 0.01	2.76 ± 0.17
Saturated	7.46 ± 0.43	7.46 ± 0.57	5.17 ± 0.24	7.43 ± 0.17	6.67 ± 0.74	3.77 ± 0.23	5.79 ± 0.16	5.00 ± 0.26	4.36 ± 0.13	6.72 ± 0.03	7.01 ± 0.48
Monounsaturated	14.9 ± 0.77	14.84 ± 1.24	10.9 ± 0.69	21.5 ± 0.58	13.8 ± 1.05	6.64 ± 0.25	13.2 ± 0.61	8.86 ± 1.26	5.27 ± 0.16	13.1 ± 0.05	15.1 ± 0.68
Polyunsaturated	7.06 ± 0.22	7.06 ± 2.47	12.8 ± 0.17	11.3 ± 0.1	13.6 ± 1.79	7.95 ± 0.26	13.7 ± 0.84	13.3 ± 1.45	9.48 ± 0.19	15.4 ± 0.38	9.18 ± 0.36
Total lipids	30.4 ± 2.4	30.38 ± 4.28	29.7 ± 1.8	41.4 ± 1.1	35.4 ± 5.8	18.9 ± 0.4	33.6 ± 0.58	27.8 ± 4.9	19.9 ± 0.69	36.3 ± 0.75	32.3 ± 1.5

The values are expressed as average and standard deviation ($\mu \pm \sigma$) for $n = 2$

Significant differences ($p < 0.05$) between seawater control (0 day, − 1.87 °C) and a specific condition are indicated as gray cells

Saturated = sum of 14:0, 16:0, 18:0

Monounsaturated = sum of 16:1$n-7$, 18:1$n-7$, 16:1$n-9$, 18:1$n-9$, 20:1$n-9$, 22:1$n-9$

Polyunsaturated = sum of 18:2$n-6$, 20:4$n-6$, 18:3$n-3$, 20:5$n-3$, 22:5$n-3$, 22:6$n-3$

at 100 °C for 5 min, and cooled in cold water. Subsequent aliquots of hexane (2 mL) and water (2 mL) were added, vortex-mixed, centrifuged at 1207×*g* for 1 min, and the hexane phase collected. An additional 2 mL aliquot of hexane is added to the remaining water, vortex-mixed, centrifuged at 1207×*g* for 1 min, and the hexane phase pooled together with the initial collection. Depending on the fat content, the sample is either concentrated under nitrogen or diluted with hexane and subsequently subjected to gas chromatography analysis.

Instrument

A Perkin-Elmer Autosystem XL, equipped with a liquid autosampler and a flame ionization detector was used. The FAME samples were analyzed on a CP-Sil 88 capillary column (50 m, 0.32 mm ID, 0.2 µm film thickness; Varian, Courtaboeuf, France). Data collection was performed by the Perkin−Elmer Chromeleon® version 7. The analysis time was 60 min and the temperature program was as follows: the oven temperature was held at 60 °C for 1 min, ramped to 160 °C at 25 °C per min, held at 160 °C for 25 min, ramped to 220 °C at 3 °C per min, held at 220 °C for 10 min. Direct on−column injection was used. The injector port temperature was ramped instantaneously from 50 to 250 °C and the detector temperature was 250 °C. The carrier gas was ultrapure helium at a pressure of 80 kPa.

Data analysis

The impact of the acclimation period on the fatty acid profile from liver of *T. bernacchii* was evaluated by comparing the variance components at 0 and 30 days. After 30 days of acclimation, the effect of time (0, 1, 5, and 10 days) and temperature (− 1.8, 0.0, 1.0 and 2.0 °C) on the levels of fatty acids in liver of *T. bernacchii* were evaluated by using the experimental arrangement described in Table 1. The variables were modeled according to a quadratic polynomial of the form:

$$y = b_o + b_1x_1 + b_2x_2 + b_{12}x_1 \times x_2 + b_1^2x_1^2 + b_2^2x_2^2 \quad (1)$$

where y represents the concentration of fatty acid in mg g^{-1}, the terms x_1 and x_2 represent the variables time and temperature, respectively, the term b_o represents the intercept, b_1 and b_2 are the linear coefficients, b_{12} is the first-order interaction effect coefficient, and $b_1^2 b_2^2$ are second-order curvature effect coefficients. A detailed description of the levels of the variables time and temperature is given in Table 1. Before modeling the various fatty acids, their normality was evaluated by comparing the values of the experimental distribution with the corresponding points of the normal distribution using inverse normal plots (Kirkwood and Sterne 2003). Briefly, the inverse normal values (inv) are calculated by arranging the experimental means (μ) in increasing order and the value of the standard normal distribution corresponding to its quantile (aka *probit*) is multiplied by the corresponding standard deviation (σ) by the expression:

$$inv = \mu + probit \times \sigma \quad (2)$$

The original $\mu \pm \sigma$ values are plotted against the computed *inv* values and the inverse normal graphs are generated by using Microsoft Excel 2013 (Online Resource 3).

A comprehensive explanation of the different steps involved in the development of the mathematical model for stearic acid (18:0) is given in Fig. 2. The various features of Fig. 2 are described by using roman numerals. The first step consisted of running in random order the experimental matrix, (i) which contains duplicate conditions for time (x_1) and temperature (x_2), and determining the responses ($y_{18:0}$) which are expressed in mg g^{-1} of fatty acid. (ii) Secondly, the modeling process is carried out by constructing a design matrix (iii) with a number of columns (6 columns) equivalent to the number of coefficients in Eq. 1 (6 coefficients). The six columns in (iii) are constructed as follows: the first column (x_0) is filled in with a numerical value of 1 and it is used to estimate the intercept (b_0). The two subsequent columns (in gray), filled in with the experimental values of time (x_1) and temperature (x_2), are used to determine the coefficients b_1 and b_2. The fourth column (x_{12}) is the result of multiplying columns x_1 and x_2 and allows computing the coefficient b_{12}. The last two columns (x_1^2 and x_2^2) represent the square of the columns x_1 and x_2 and they are used to calculate the coefficients $b_1^2 b_1^2$ and b_2^2. The vector $y_{18:0}$ (ii) is regressed on the matrix (iii) and equation (iv) is computed. The validity of (iv) is confirmed by using the ratio between the lack-of-fit mean square and the pure error mean square at a 95% confidence level (Online Resource 4) and described elsewhere (Araujo 2009). After validating model (iv), a matrix of estimated responses (v) is determined within the boundaries of the experimental ranges by substituting in (iv) the variables x_1 and x_2 (between 0 and 10 days and between − 2 and 2 °C, respectively). Matrix (v), for example, indicates that the concentration of 18:0 at 0-day/2 °C (0.59 mg g^{-1}) is lower than 10-days/2 °C (1.21 mg g^{-1}). To rapidly summarize the large amount of data in matrix (v), a graphical display of (vi) can be used. The graphical display in Fig. 2 is more practical and operable and shows the distribution of data through color change. Every color in (vi) represents areas with similar concentrations. For example, according to (vi), the brown and gray colors indicate concentration ranges of 0.5–1.0 mg g^{-1} and 1.0–1.5 mg g^{-1}, respectively. In addition, a rapid inspection of (vi) allows concluding that condition 0-day/2 °C exhibits a lower concentration than condition 10-days/2 °C, without the need of a detailed examination of the numerical matrix (iv). The visualization of the computed regressions for the different fatty acids was

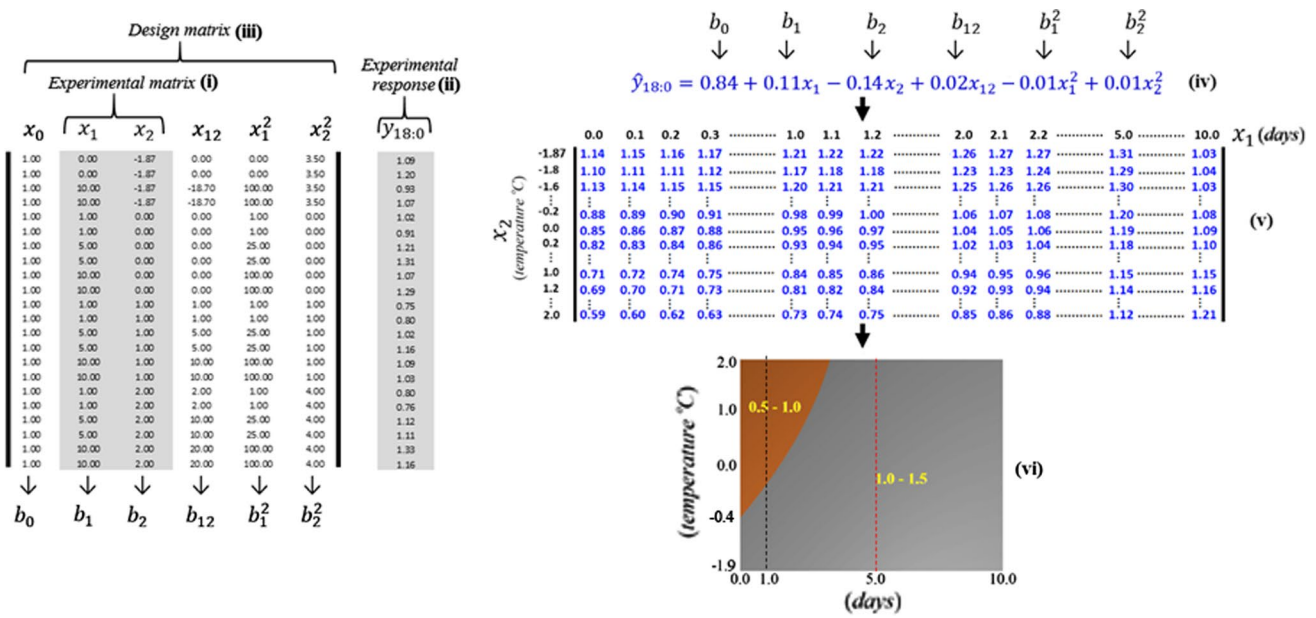

Fig. 2 Steps involved in the development of the mathematical model for stearic acid (18:0). The experimental matrix (i) is proposed and the corresponding responses (ii) are obtained. A design matrix (iii) is constructed and the equation (iv) is computed by regressing (ii) on (iii). The validity of (iv) is confirmed statistically (see Online Resource 4) and a matrix of estimated responses (v) is generated within the experimental limits of the variables x_1 (from 0.0 to 10.0 days) and x_2 (from −1.9 to 2.0 °C). The large amount of data in (v) is plotted and summarized in (vi). The colors in (vi) represent areas with similar concentrations and they are read as follows: if a black dashed line is drawn at day 1 between −1.9 and 2.0 °C, then moving up along this black line allows estimating a concentration range of 1.0–1.5 mg g^{-1} (gray color) between −1.9 and −0.4 °C and a concentration range of 0.5–1.0 mg g^{-1} (brown color) between −0.4 and 2.0 °C. Similarly, the red dashed line at day 5 and between −1.9 and 2.0 °C allows estimating only one concentration range of 1.0–1.5 mg g^{-1} (gray color). Consequently, it is concluded that between 5 and 10 days, the concentration of 18:0 remains constant in the range of 1.0–1.5 mg g^{-1} in the whole range of explored temperatures

performed by using Microsoft Excel 2013. Statgraphics Centurion XVI (Version 16.1.11, StatPoint Technologies, Inc., Warrenton, VA, USA) was used to evaluate statistically the differences between the means of the control seawater and the acclimatized groups at different temperatures and times. Dunnett's *t* test was employed as correction procedure for comparing the various groups with the seawater control.

Results

The length and weight of the fish were computed throughout the course of the experiments. These parameters for the control seawater at 0 day were similar to those measured at different times and temperatures. Hence, the effect of allometry was negligible (Online Resource 5).

The average and standard deviation of the fatty acid profiles of the caught fish in the Ross sea (control seawater) and the fish acclimatized for 30 days in a circulating seawater tank were determined (Table 1) and the ratios of the between/within variance components for these profiles (aka Fisher ratio or *F* test) computed and compared against the tabulated *F* value of 18.51 ($p = 0.05$) for 1 and 2 degrees of freedom in the numerator and denominator, respectively. The results revealed the absence of statistical significant differences between the two types of controls (Online Resource 6).

The fatty acid profiles from liver of *T. bernacchii* at different times (1, 5, and 10 days) and temperatures (0, 1, and 2 °C) after the acclimation period are described in Table 1. The oleic acid (18:1$n-9$) and docosahexaenoic acid (22:6$n-3$) exhibited the highest concentrations in Table 1, followed by palmitic oleic acid (16:1$n-7$) and eicosapentaenoic acid (20:5$n-3$) (9.16 ± 0.39, 8.60 ± 0.34, 8.32 ± 0.42, and 5.18 ± 0.16 mg g^{-1}, respectively). The inverse normal plots (obtained by using Online Resource 3) revealed a high degree of linearity for the various fatty acids (Online Resource 7) confirming that the data in Table 1 were normally distributed. Quadratic polynomial models were generated by using Eq. 1 and the data in Table 1. The behavior of the saturated fatty acids (SFA) (14:0, 16:0, 18:0), monounsaturated fatty acids (MUFA) (16:1$n-7$, 18:1$n-7$, 16:1$n-9$, 18:1$n-9$, 20:1$n-9$, 22:1$n-9$), and polyunsaturated fatty acids (PUFA) (18:2$n-6$, 20:4$n-6$, 18:3$n-3$, 20:5$n-3$, 22:5$n-3$, 22:6$n-3$) (mg g^{-1}) as a function of the variables time (days) and temperature (°C) is visualized in Fig. 3. The results exhibited good prediction capabilities

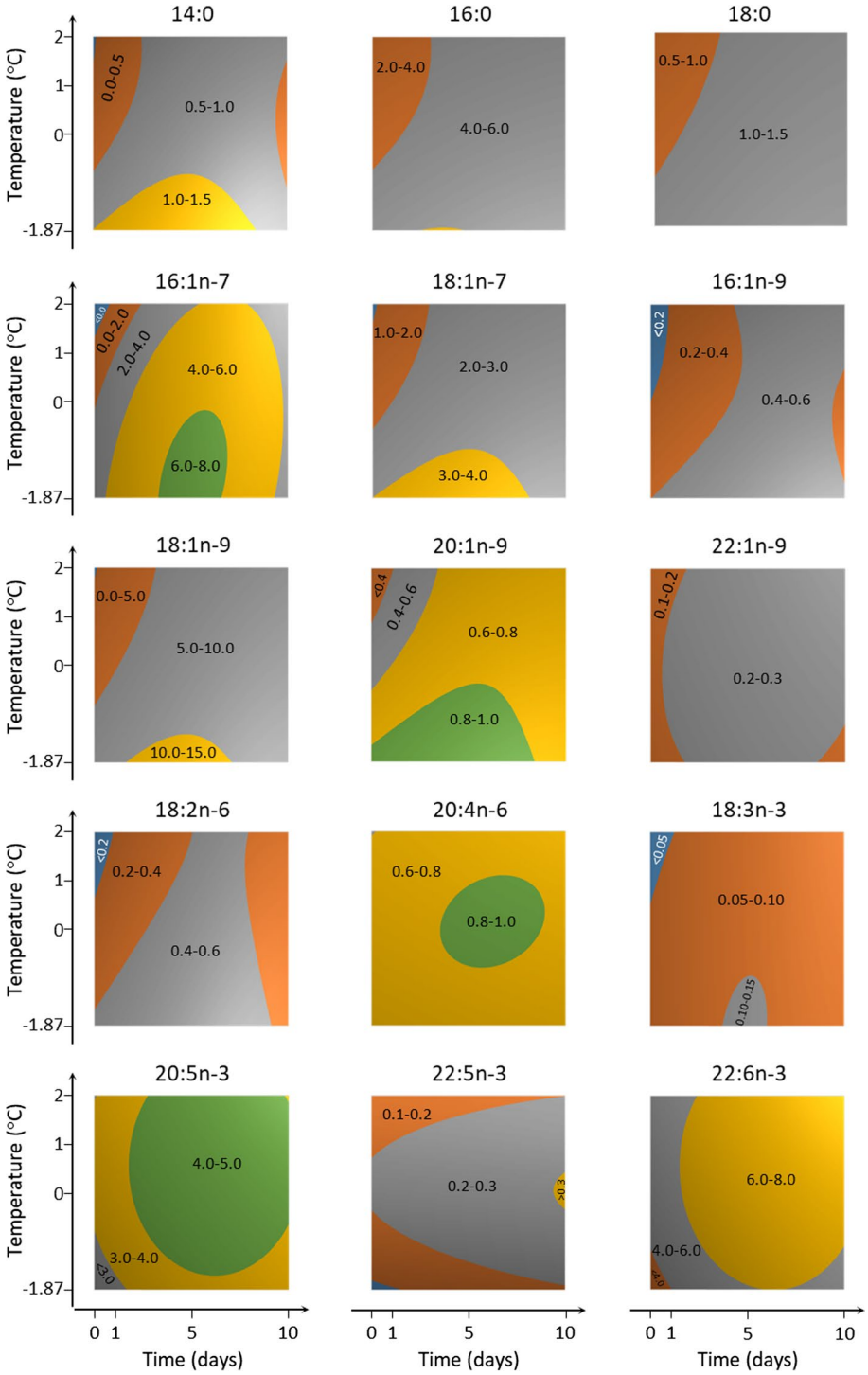

Fig. 3 Mathematical models describing the variation in mg of fatty acid per g of liver of *T. bernacchii* as a function of time and temperature (colored areas in mg g^{-1})

as observed in Tables 1, 2, where the experimental and predicted concentrations did not revealed any statistical difference between them. Consequently, if a model reproduces the concentrations at the experimental times and temperatures, then it must also reproduce any concentration at any point allocated inside the experimental ranges. The main goal of modeling environmental variables is predicting valid information from the proposed models. This very common approach is implemented in worldwide laboratories where calibration curves are prepared using a preselected concentration range and predictions are made in areas between the experimental points. The computed regression equations are described in Table 2. A variation in fatty acid concentration around 15% was indicative of change in stability. This threshold was based on a study of IUPAC (Firestone and Horowitz 1979) and the FDA

Table 2 Predicted concentrations in mg g^{-1} and regression models used for calculations. The bracketed statistical t values indicate no significant differences between predicted and experimental concentrations in Table 1 ($p=0.01$)

Days (x_1)	0	1	5	10	1	5	10	1	5	10	Regression models
°C (x_2)	−1.87	0	0	0	1	1	1	2	2	2	
14:0	0.93 (0.19)	0.46 (1.63)	0.84 (35.70)	0.40 (2.39)	0.28 (1.20)	0.76 (24.32)	0.44 (1.36)	0.21 (4.52)	0.79 (14.30)	0.59 (0.37)	$y = 0.26 + 0.22x_1 - 0.26x_2 + 0.02x_1x_2 - 0.02x_1^2 + 0.06x_2^2$
16:0	5.29 (0.18)	3.78 (0.19)	4.79 (2.22)	4.08 (1.16)	3.22 (2.07)	4.59 (3.06)	4.39 (3.12)	2.94 (2.83)	4.70 (1.63)	4.98 (0.41)	$y = 3.32 + 0.50x_1 - 0.80x_2 + 0.10x_1x_2 - 0.04x_1^2 + 0.14x_2^2$
18:0	1.14 (0.05)	0.95 (0.14)	1.19 (0.97)	1.09 (0.55)	0.84 (1.81)	1.15 (0.64)	1.15 (2.29)	0.73 (1.87)	1.12 (0.56)	1.21 (0.26)	$y = 0.84 + 0.11x_1 - 0.14x_2 + 0.02x_1x_2 - 0.01x_1^2 + 0.01x_2^2$
18:3$n-3$	0.07 (0.31)	0.07 (0.24)	0.10 (7.19)	0.07 (1.76)	0.06 (0.36)	0.09 (0.54)	0.07 (0.68)	0.05 (1.337)	0.08 (10.19)	0.06 (0.35)	$y = 0.05 + 0.02x_1 - 0.01x_2 + 0.001x_1x_2 - 0.001x_1^2 - 0.001x_2^2$
20:5$n-3$	2.41 (0.70)	3.66 (3.28)	4.67 (13.65)	4.18 (0.37)	3.68 (5.11)	4.69 (0.04)	4.21 (0.91)	3.39 (0.52)	4.42 (4.65)	3.94 (33.06)	$y = 3.21 + 0.49x_1 + 0.16x_2 + 0.002x_1x_2 - 0.004x_1^2 - 0.15x_2^2$
22:5$n-3$	0.10 (1.14)	0.22 (1.38)	0.26 (13.76)	0.30 (1.36)	0.20 (26.63)	0.24 (1.61)	0.28 (0.97)	0.13 (2.58)	0.17 (11.23)	0.20 (2.24)	$y = 0.21 + 0.01x_1 + 0.01x_2 - 0.001x_1x_2 - 0.0003x_1^2 + 0.03x_2^2$
22:6$n-3$	3.25 (0.35)	5.55 (39.00)	7.48 (17.38)	6.60 (0.49)	5.57 (6.61)	7.49 (0.14)	6.58 (0.51)	5.06 (0.55)	6.96 (4.85)	6.03 (3.44)	$y = 4.70 + 0.92x_1 + 0.29x_2 - 0.005x_1x_2 - 0.07x_1^2 - 0.26x_2^2$
18:2$n-6$	0.42 (0.10)	0.32 (1.80)	0.45 (3.13)	0.30 (1.47)	0.25 (2.06)	0.42 (1.52)	0.31 (1.07)	0.20 (1.41)	0.40 (19.71)	0.33 (0.31)	$y = 0.25 + 0.08x_1 - 0.08x_2 + 0.01x_1x_2 - 0.01x_1^2 + 0.01x_2^2$
20:4$n-6$	0.66 (0.13)	0.74 (5.46)	0.82 (10.33)	0.78 (0.66)	0.71 (23.35)	0.80 (0.73)	0.77 (0.21)	0.63 (1.86)	0.73 (6.44)	0.72 (23.08)	$y = 0.71 + 0.04x_1 - 0.01x_2 + 0.003x_1x_2 - 0.003x_1^2 - 0.02x_2^2$
16:1$n-9$	0.38 (0.88)	0.26 (1.65)	0.42 (5.27)	0.38 (0.52)	0.22 (2.95)	0.41 (10.07)	0.41 (1.70)	0.21 (2.55)	0.43 (4.10)	0.48 (0.58)	$y = 0.20 + 0.07x_1 - 0.07x_2 + 0.01x_1x_2 - 0.01x_1^2 + 0.02x_2^2$
18:1$n-9$	7.74 (0.24)	4.62 (0.36)	7.94 (3.18)	5.07 (1.07)	2.93 (1.10)	7.04 (1.90)	5.19 (1.61)	1.70 (2.48)	6.61 (16.05)	5.77 (2.53)	$y = 3.01 + 1.76x_1 - 2.12x_2 + 0.20x_1x_2 - 0.16x_1^2 + 0.23x_2^2$
20:1$n-9$	0.84 (0.03)	0.60 (1.90)	0.77 (3.13)	0.65 (0.96)	0.48 (5.38)	0.72 (1.52)	0.69 (0.53)	0.39 (2.42)	0.69 (19.57)	0.74 (0.62)	$y = 0.52 + 0.09x_1 - 0.15x_2 + 0.02x_1x_2 - 0.01x_1^2 + 0.01x_2^2$
22:1$n-9$	0.17 (0.06)	0.21 (29.88)	0.25 (3.24)	0.22 (1.21)	0.20 (3.21)	0.25 (1.41)	0.23 (0.60)	0.18 (0.25)	0.24 (2.12)	0.22 (0.20)	$y = 0.19 + 0.02x_1 - 0.003x_2 + 0.001x_1x_2 - 0.002x_1^2 - 0.005x_2^2$
16:1$n-7$	3.28 (1.24)	3.09 (1.35)	5.82 (5.94)	3.75 (0.21)	1.86 (0.44)	5.02 (12.45)	3.47 (1.74)	0.27 (15.86)	3.84 (23.09)	2.81 (2.50)	$y = 1.80 + 1.42x_1 - 1.15x_2 + 0.10x_1x_2 - 0.12x_1^2 - 0.19x_2^2$
18:1$n-7$	2.88 (0.06)	1.99 (0.54)	2.69 (0.00)	2.24 (0.98)	1.59 (2.45)	2.51 (0.14)	2.35 (1.94)	1.33 (1.26)	2.48 (1.46)	2.60 (0.89)	$y = 1.66 + 0.35x_1 - 0.53x_2 + 0.06x_1x_2 - 0.03x_1^2 + 0.07x_2^2$

recommendations (FDA 2013) that acknowledged 15% as the maximum variation for fatty acid analysis.

Saturated fatty acids

The regression models for 14:0, 16:0, and 18:0 (Table 2) indicate that at 0 and 1 days, there were a continuous decrease in the concentrations of SFA between − 1.8 and 2.0 °C (Fig. 3). For example, the concentration of 16:0 decreases by 58% and ~ 49% at 0 and 1 day, respectively, while 18:0 was characterized by a continuous reduction in concentration when the temperature was raised from − 1.8 to 0.0 °C (~ 2 5%), 1.0 °C (~ 40%), and 2.0 °C (~ 50%) at 0 and 1 day (Fig. 3). These percentages of reduction are computed by means of the regression models of SFA in Table 2. The behavior at 5 days for 14:0 between − 1.8 and 0.0 °C was characterized by a decrease in concentration around 35% with an additional reduction of approximately 10% when the temperature was increased from 0.0 to 1.0 °C with no further change in concentration (0.77 ± 0.02 mg g^{-1}) between 1.0 and 2.0 °C. A similar reduction in concentration (~ 40%) was observed at 10 days between − 1.8 and 1.0 °C. At 5 and 10 days, the concentrations of 16:0 (5.01 ± 0.64 and 4.42 ± 0.37 mg g^{-1}, respectively) and 18:0 (1.16 ± 0.09 mg g^{-1}) remain almost constant in the whole range of temperatures as reflected in the contour plot in Fig. 3, where a monochromatic gray region between 5 and 10 days indicates a concentration range that varies between 4.0 and 6.0 mg g^{-1} for 16:0 or between 1.0 and 1.5 mg g^{-1} for 18:0. Total SFA contents in liver of fish exposed to higher temperatures for 1 day (5.26 ± 0.35, 3.82 ± 0.29, and 5.09 ± 0.36 mg g^{-1} at 0.0, 1.0, and 2.0 °C, respectively) were lower than the seawater control group (7.57 ± 0.63 mg g^{-1}). Table 1 showed that between 5 (~ 6.7 ± 0.29 mg g^{-1}) and 10 days (6.23 ± 0.64 mg g^{-1}), the total SFA contents were similar to the control group.

Monounsaturated fatty acids

At 0, 1, and 5 days, there were significant reductions in the concentration of vaccenic acid (18:1n − 7), palmitic oleic acid (16:1n − 7), oleic acid (18:1n − 9), and gondoic acid (20:1n − 9) (Fig. 3). Table 1 and the regression models (Table 2 and Fig. 3) allow estimating a maximum reduction in the concentrations of 18:1n − 7, 16:1n − 7, 18:1n − 9, and 20:1n − 9 of approximately 60, 92, 80, and 60%, respectively, at 1 day and between − 1.8 and 2.0 °C. The behavior of 16:1n − 9 was characterized by a progressive reduction in concentration of approximately 50, 60, and 70% at 0 day and 40, 50, and 55% at 1 day when the temperature was increased at 0.0, 1.0, and 2.0 °C, respectively. Between 5 and 10 days, the concentration of the majority of MUFA remains almost constant in the whole range of temperatures as observed in Fig. 3, where monochromatic regions indicate similar concentrations. For example, a statistical comparison at the 95% confidence level of the lowest (0.34 ± 0.05 mg g^{-1}) and highest (0.51 ± 0.06 mg g^{-1}) recorded concentrations of 16:1n − 9 at 10 days (Table 1) revealed the lack of significant differences for this fatty acid at different temperatures ($t_{experimental} = 2.18$ vs. $t_{tabulated} = 4.30$). Total MUFAs showed a significant reduction with respect to the seawater control group after the first day of exposure to higher temperature (2.0 °C) (~15 ± 0.77 and 5.27 ± 0.16 mg g^{-1}, respectively) (Table 1).

The results did not reveal significant changes in the concentration of 22:1n − 9 in the whole ranges of time and temperature (0.21 ± 0.03 mg g^{-1}) as reflected in Fig. 3 where the response surface displays basically one single color (gray). A statistical comparison of the lowest and highest concentrations of 22:1n − 9 in Table 1 (0.17 ± 0.01 and 0.28 ± 0.05 mg g^{-1}, respectively) unveiled an experimental t value of 2.16 that was much lower than the tabulated 4.30 at 2 degrees of freedom. Total MUFAs showed a significant reduction with respect to the seawater control group after the first day of exposure to higher temperature (2.0 °C) (~ 15 ± 0.77 and 5.27 ± 0.16 mg g^{-1}, respectively) (Table 1).

Polyunsaturated fatty acids

The elevated temperatures led to the observed variable responses of PUFA concentrations (Fig. 3). Linoleic acid (18:2n − 6) revealed a similar reduction in concentrations at 0 and 1 day in the whole range of tested temperatures. The concentration remains constant (0.49 ± 0.06 mg g^{-1}) between 0 and 5 days at − 1.8 °C with a further decreased in concentration of around 30% at 10 days. It was also observed that at 5 and 10 days, the concentration of 18:2n − 6 was independent of the temperature with average concentration values of 0.46 ± 0.07 and 0.32 ± 0.02 mg g^{-1}, respectively. The model for arachidonic acid (20:4n − 6) did not show significant changes in the concentration at different days and temperatures as reflected within day and within temperature averages. The former averages for 20:4n − 6 were 0.66 ± 0.05, 0.69 ± 0.05, 0.77 ± 0.05, and 0.73 ± 0.06 mg g^{-1} at 0, 1, 5, and 10 days, while the latter were 0.67 ± 0.03, 0.76 ± 0.04, 0.74 ± 0.06, and 0.67 ± 0.07 mg g^{-1} at − 1.8, 0.0, 1.0, and 2.0 °C, respectively. In addition, the concentration of linolenic acid (18:3n − 3) was independent of the temperature at 0 day (0.06 ± 0.01 mg g^{-1} between − 1.8 and 0.0 °C), 1 day (0.07 ± 0.01 mg g^{-1} between − 1.8 and 1.0 °C), 5 days (0.09 ± 0.01 mg g^{-1} between 0.0 and 2.0 °C), and 10 days (0.06 ± 0.00 mg g^{-1} between − 1.8 and 2.0 °C). Out of the specified temperature ranges for 0 and 1 day, there was a decrease in concentration while for 5 days, there was an increase in the concentration.

Eicosapentaenoic acid (20:5$n-3$), docosapentaenoic acid (22:5$n-3$), and docosahexaenoic acid (22:6$n-3$) were characterized by a general increase in concentration at different temperatures and days. The concentration of 20:5$n-3$ increased ~30% between 0 and 10 days when the temperature was raised from −1.8 to 0.0 °C. Between 0.0 and 2.0 °C, the average concentrations at 0, 1, 5, and 10 days were 3.14 ± 0.15, 3.58 ± 0.15, 4.60 ± 0.14, and 4.12 ± 0.13 mg g^{-1}, respectively. Increases in the concentration of 22:5$n-3$ of 18% (between 0 and 1 day), 54% (between 1 and 5 days), and 33% (between 5 and 10 days) were observed at −1.8 °C. Identical increase patterns in concentration of 6% (0–1 day), 20% (1–5 days), and 15% (5–10 days) were observed at 0.0 and 1.0 °C. At 2.0 °C, the increase pattern was 9% (0–1 day), 29% (1–5 days), and 19% (5–10 days). The response surface for 22:6$n-3$ displayed a behavior similar to 20:5$n-3$ (Fig. 3). There was an average increase in concentration of 38% in the whole range of time (0–10 days) between −1.8 and 0.0 °C. Between 0.0 and 2.0 °C, the average concentrations at 0, 1, 5, and 10 days were 4.56 ± 0.26, 5.41 ± 0.26, 7.32 ± 0.28, and 6.41 ± 0.30 mg g^{-1}, respectively. Total PUFA showed a statistically significant increase after 1 day of exposure for all tested temperatures that persisted until 5 days of exposure to higher temperatures (Table 1).

Discussion

The ubiquitous species of stenothermal are characterized by their evolutionary adaptation to the extremely low temperatures of the Antarctic region, which is associated with a unique physiological adaptation but as well involves limited compensation capacities to the environmental changes. These species cope with the cold environment by several types of adaptations including, a reduction or loss of hemoglobin, higher mitochondrial densities, expression of antifreeze glycoproteins, lack of heat shock response, variation in lipid class or changes in the FA composition (Eastman 1993; Pörtner and Playle 1998). The present research aims to answer a crucial question of ocean warming and its consequences on ecological interactions, by measuring changes in fatty acid profiles over time with variation in body temperature of Antarctic fish *T. bernacchii*.

The models for 14:0, 16:0, and 18:0 revealed that at 10 days and between 0.0 and 2.0 °C, there were non-significant increases of approximately 4, 5, and 6% in absolute concentrations, respectively (Fig. 3). These increases in concentrations are equivalent to 14, 16, and 16% in relative units (computed from Online Resource 8), respectively. These results are close to those reported by Brodte et al. (2008) who measured the fatty acid profiles in liver of Antarctic fish (*Pachycara brachycephalum*) and reported non-significant increases in concentrations of 20, 16, and 25% for 14:0, 16:0, and 18:0 (measured in relative units), respectively, at 120 days of acclimation and between 0.0 and 2.0 °C. Similarly, non-significant increments of 15 and 20% in relative concentration have been observed in temperate fish by Roche and Pérès (1984) and Hazel (1979), respectively. In the present study, the content of SFA in the liver of the control group at −1.87 °C was 7.46 ± 0.43 mg g^{-1} corresponding to 24.0% of total FA. The obtained value for hepatic SFA was comparable to those observed in *T. bernacchii* in other studies (Corsolini and Borghesi 2017; Malekar et al. 2018). Upon higher temperatures, the relative concentration of SFA remained almost constant in the liver of *T. bernacchii,* over the studied acclimation period (1–10 days). Similarly, Brodte et al. (2008) found that the content of SFA in the liver of Antarctic fish did not change at higher temperatures (2, 4, and 6 °C) when compared to the control group (−0 °C). Recently, Malekar et al. (2018) demonstrated also that the SFA composition of phospholipids in the liver of *T. bernacchii* remained constant at elevated temperatures during 14 days. The same authors demonstrated that increasing the temperature to 6 °C resulted in an increase of saturated fatty acids and a decrease in the content of unsaturated fatty acids in the membrane of *T. bernacchii* liver. Based on the present and previous studies, the variation of SFA composition in response to changes in temperatures seems to occur differently based on the temperature of exposure (Brodte et al. 2008; Malekar et al. 2018).

The observed decrease in concentration for 16:1$n-7$ between 0 and 5 days as the temperature increases has been also reported in different polar and temperate fish species by evaluating the effect of increasing the temperature at specific times (Roche and Pérès 1984; Dey et al. 1993; Brodte et al. 2008). The reduction in concentration for 18:1$n-7$ and 18:1$n-9$ between 0 and 5 days in the investigated temperature range is in agreement with the results of Dey et al. (1993) and Roche and Pérès (1984), who reported a decreasing in concentration of 18:1n (not distinction was made between the two isomers) in temperate fish after comparing two experimental temperatures. Brodte et al. (2008) reported an astonishing increase in concentration of ~3000% for 18:1$n-7$ in the liver of Antarctic fish (difference between fish exposed to 0.0 and 4.0 °C during 120 days) and a decrease of 39% in temperate fish (difference between fish exposed to 4.0 °C and 6.0 °C during 120 days). The behavior of 16:1n in livers of different temperate fish has been analyzed after one month of acclimation period at two different temperatures (Roche and Pérès 1984; Dey et al. 1993). The results of these studies did not reveal differences in FA concentrations in seawater fish (Roche and Pérès 1984) but in freshwater fish (Dey et al. 1993). Similar results to those reported for seawater fish have been predicted by the model for 16:1$n-9$ (Fig. 3) where the concentration does

not change significantly between 5 and 10 days in the range of − 1.8 and 2.0 °C. The predicted constant concentration at 10 days and between 0.0 and 2.0 °C by the 20:1*n* − 9 model (Fig. 3) for Antarctic fish *T. bernacchii* are in full agreement with the studies on Antarctic fish *Pachycara brachycephalum* which did not observe change in concentration after 120 days in the range of 0.0 and 2.0 °C (Brodte et al. 2008).

The contour plots for the six PUFA (18:2*n* − 6, 20:4*n* − 6, 18:3*n* − 3, 20:5*n* − 3, 22:5*n* − 3, 22:6*n* − 3) predict non-significant changes in concentration at 1, 5, and 10 days between 0.0 and 2.0 °C. The predictions are consistent with the report of Brodte et al. (2008) who determined these PUFAs in liver from Antarctic fish without any significant change in concentration in the same range of temperatures (0.0 and 2.0 °C) after 120 days. Studies on temperate fish have not observed change in the concentration of 18:2*n* − 6, 20:4*n* − 6, 18:3*n* − 3, 20:5*n* − 3, 22:5*n* − 3, and 22:6*n* − 3 (Hazel 1979; Roche and Pérès 1984; Dey et al. 1993; Brodte et al. 2008).

In their natural environment, marine organisms adapt their physiological function to cope with different stress factors. There is an inverse correlation between ambient temperature and the content of unsaturated fatty acids (Roche and Pérès 1984; Hazel 1990, 1995). Indeed, the cell membranes of fish adapted to live in cold temperatures contained generally high levels of MUFA, in particular 18:1*n* − 9, which reflect the ability to maintain a proper fluidity of the biological membrane (Sinensky 1974; Hazel and Carpenter 1985; Tiku et al. 1996). The biochemical response of stenothermal species to lower temperature is an increase in the concentration of unsaturated fatty acids in both the membrane and depot of lipids (Brodte et al. 2008; Malekar et al. 2018; Truzzi et al. 2018a, b). In the present study, when fish were immediately exposed to higher temperature (2.0 °C after 1 day of exposure), the concentration of MUFA decreased in the liver of *T. bernacchii*, specifically the concentration of oleic acid (18:1*n* − 9) decreased by 50% compared to the control group (− 1.8 and 2.0 °C after 1 day of exposure). Such effect has been observed previously in many fish species including *Pachycara brachycephalum*, *Zoarces viviparus* (Brodte et al. 2008) and *Salmo gairdneri* (Hazel 1979; Sellner and Hazel 1982). However, those results were observed at different temperatures and exposure times (from 3 days to 3 months) and in organs (liver, muscle, gill, and kidney) of different fish species (Smith and Kemp 1971; Sellner and Hazel 1982; Hazel and Carpenter 1985), whereas for *T. bernacchii*, the change in hepatic FA was observed immediately after 1 day of exposure to higher temperatures. The decrease in MUFA composition in the liver of *T. bernacchii* was in line with the inverse relation between unsaturated fatty acids and changes in temperature, as it has been demonstrated in different fish species (Hazel 1979; Sellner and Hazel 1982; Logue et al. 2000). Furthermore, this decrease in MUFA composition might also serve as indicator for protection mechanisms against oxidative stress induced by higher temperature, as previous studies have shown (Crockett 2008; Vinagre et al. 2014; Machado et al. 2014). Conversely, the content of PUFA in the liver of *T. bernacchii* increased after the first day of exposure to higher temperatures, from 23% of total FA (− 1.87 °C) to 36, 43, and 40% of total FA at 0.0, 1.0, and 2.0 °C, respectively. These results are somewhat surprising, since most of the studies reported a decrease in PUFA composition at higher temperatures in the tissues of different fish species. (Hazel 1979, 1990; Tiku et al. 1996; Pernet et al. 2007). However, data provided by Brodte et al. (2008) demonstrated also an increase in PUFA levels by more than 70% in the liver of Antarctic fish upon higher temperature (from 0.0 to 4.0 °C after 120 days of exposure). In this study, the authors concluded that the polar fish have already optimized their metabolism by using preferentially MUFA over the PUFA, when the temperature changes (Brodte et al. 2008). Moreover, in the present study, the effect of increasing the temperature on the FA profile of the liver was investigated after 1, 5, and 10 days, however, most of the studies reported a decrease in the PUFA composition at higher temperatures after a long period of exposure (Smith and Kemp 1971; Sellner and Hazel 1982; Hazel and Carpenter 1985). Thus, in the present study, the effect seen on the PUFA composition might be not related to changes in temperature, and also a long period of exposure to higher temperatures should be considered.

The statistical comparison ($p < 0.05$) between the seawater control (0 day, − 1.87 °C) and 1, 5, and 10 days at 0.0, 1.0, and 2.0 °C allows concluding that at 10 days of exposure to 0.0, 1.0, and 2.0 °C, the FA composition in the liver of *T. bernacchii* were similar to the control group as indicated in Table 1. Indeed, FA composition in the liver of Antarctic fish contained the same concentration as in their natural environment (− 1.87 °C). *T. bernacchii* evolved during the Palaeocene period, characterized by high fluctuations in temperatures, and only after this period, the temperatures in the Antarctic have decreased until the current stable temperatures (Clarke and Johnston 1996; Seebacher et al. 2005). The plasticity of these species seems to be comparable in magnitude with eurythermal and temperate fishes (Seebacher et al. 2005; Podrabsky and Somero 2006; Bilyk and DeVries 2011; Bilyk et al. 2012). Furthermore, previous research on warm acclimation of notothenioid fish demonstrated that these species have conserved the capacity to compensate for temperatures changes, by different mechanisms including astounding plasticity in cardiovascular response, metabolic control, Na^+/K^+-ATPase activity, hypo-osmoregulation, induction of the expression of many genes associated with lipid biosynthesis, or increase in their critical thermal maximum (Bilyk et al. 2012; Brauer et al. 2005; Seebacher et al. 2005; Huth and Place 2016). The

present results may indicate that *T. bernacchii* exposed to thermal shock tries to acclimate to the elevated temperatures in order to maintain the functionally to the liver. Furthermore, there were no detrimental health effects on these fish species when exposed to higher temperatures up to 4.0 °C during several weeks (Bilyk and DeVries 2011). Thus, it can be hypothesized that the changes in fatty acids composition observed in the liver of *T. bernacchii* might reflect a shift towards a normal acclimation response, rather than symptoms of heat stress.

Conclusions

The fact that the computed models for Antarctic *T. bernacchii* predict changes that are in agreement with well-documented studies not only on polar but also on temperate fish might indicate that polar and temperate teleosts will respond in a similar way to global warming, which in turn might indicate the existence of a common underlying model for teleosts.

To the best of our knowledge, this is the first report modeling the changes in fatty acid concentration as a function of systematic and simultaneous variations in time and temperature.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving *Trematomus bernacchii* were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

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