

# Contribution of sea ice organic matter in the diet of Antarctic fishes: a diatom-specific highly branched isoprenoid approach

A. Goutte · Y. Cherel · C. Ozouf-Costaz ·  
C. Robineau · J. Lanshere · G. Massé

Received: 21 August 2013/Revised: 22 January 2014/Accepted: 12 March 2014/Published online: 26 March 2014  
© Springer-Verlag Berlin Heidelberg 2014

**Abstract** New sets of diatom-specific biomarkers, highly branched isoprenoids (HBIs), have been recently proposed to trace carbon flow from ice algae and pelagic phytoplankton to higher trophic level organisms. In the Antarctic, diene, a HBI of sea ice origin was more abundant in ice-associated species, while triene, a HBI of phytoplanktonic origin, was more abundant in pelagic species. However, this HBI approach has never been applied on Antarctic benthic species. Here, we analyzed diene and triene in the liver and the muscle of eight Antarctic coastal fish species (108 specimens). HBI lipids were detected in all specimens, confirming the contribution of sea ice and pelagic organic matter in coastal benthic fish species. Moreover, HBI markers were much more concentrated in the liver than in white muscle, and the relative concentrations of diene and triene strongly varied among species, as a probable result of species differences in feeding habits and trophic ecology. Seasonal variations in HBI concentrations were detected during the whole year in white muscle, but not in the liver. These findings are consistent

with the well-known spring bloom in November–December, just before the annual ice break up, and the second proliferation of ice algae during the land-fast ice formation, in April–May. Therefore, investigation of HBI lipids in white muscle will likely shed new light on seasonal changes in the contribution of ice algal-derived organic matter in higher trophic level organisms.

**Keywords** Benthic · Ice proxy · Habitat · Pelagic · Southern Ocean · Sea ice

## Introduction

The use of biogeochemical tracers, such as stable isotopes, fatty acids, DNA tracers or pollutants greatly, contributes understanding trophic interactions and feeding ecology of species. Recently, new sets of diatom-specific biomarkers have been proposed to trace carbon flow from ice algae and pelagic phytoplankton to higher trophic level organisms in the Arctic and the Antarctic marine environments (Brown and Belt 2012a, b; Brown et al. 2012, 2013a, b; Goutte et al. 2013). Polar marine ecosystems are sustained by the proliferation of sympagic (e.g., ice-associated) algae at the bottom of the sea ice and the bloom of pelagic phytoplankton during the summer ice decay (Garrison 1991; Lizotte 2001; Arrigo and Thomas 2004). These two sources of primary production are either incorporated in pelagic trophic webs or assimilated by benthic organisms after sedimentation.

Estimating the relative contribution of ice algal-derived organic matter in polar marine consumers remains a challenging task, due to the lack of specific biomarkers. In that respect, the so-called highly branched isoprenoids (HBIs) have demonstrated great potential as fingerprints of ice

---

A. Goutte (✉) · C. Robineau · J. Lanshere · G. Massé  
LOCEAN/IPSL, Université Pierre et Marie Curie, 75005 Paris,  
France  
e-mail: agoutte@gmail.com

A. Goutte · Y. Cherel  
Station d'Ecologie de Chizé-La Rochelle, UMR 7372  
CNRS-Université de la Rochelle, 79360 Villiers en Bois, France

C. Ozouf-Costaz · J. Lanshere  
Museum National d'Histoire Naturelle, CNRS UMR 7138,  
75231 Paris Cedex 05, France

G. Massé  
CNRS and Université Laval, UMI 3376, Takuvik, Quebec, QC,  
Canada

algae versus pelagic phytoplankton (Belt et al. 2007; Massé et al. 2011). Indeed, HBI lipids are produced by a restricted number of diatoms, including sympagic, pelagic, benthic and freshwater diatoms (Nichols et al. 1988; Volkman et al. 1994; Johns et al. 1999; Sinninghe Damsté et al. 1999; Belt et al. 2001a, b, c, 2007). In the Arctic, ice proxy with 25 carbon atoms (IP<sub>25</sub>), a mono-unsaturated HBI isomer, has been shown to be specifically produced by sea ice diatoms (Belt et al. 2007), while diene, a di-unsaturated HBI isomer, has been isolated in Antarctic sea ice diatom communities, but not in pelagic phytoplankton (Nichols et al. 1988; Johns et al. 1999; Sinninghe Damsté et al. 1999; Massé et al. 2011). Moreover, the carbon isotopic composition of diene in Antarctic sea ice ( $\delta^{13}\text{C}$ :  $-8.5$  and  $-5.7$  ‰, Massé et al. 2011) and sediments ( $\delta^{13}\text{C}$ :  $-9.1$  and  $-9.4$  ‰, Sinninghe Damsté et al. 2007) is consistent with a sea ice origin. Other polyunsaturated HBI lipids, especially a tri-unsaturated HBI isomer, hereafter triene, have been detected in phytoplankton samples in open-ocean and at the sea ice edge in Antarctica, but were absent from sea ice samples (e.g., Massé et al. 2011). The stable isotopic composition of triene in Antarctic phytoplankton samples and sediments supports its phytoplanktonic origin ( $-40$  ‰, Massé et al. 2011).

Within an ecosystem context, recent studies have shown that these HBI markers were useful for evaluating the contribution of organic matter derived from ice algae and phytoplankton in higher trophic level organisms. HBI biomarkers are transferred across trophic webs, since they were detected in a broad spectrum of consumers, including zooplankton, krill, benthic macrofauna, fishes, seabirds and marine mammals (Brown and Belt 2012a; Brown et al. 2012, 2013a, b; Goutte et al. 2013). Moreover, the concentrations of diene relative to triene (hereafter D/T ratio) were consistent with the feeding ecology and habitats of Antarctic consumers (Goutte et al. 2013). For instance, the cryopelagic fish *Pagothenia borchgrevinki* exhibited higher D/T ratios than the pelagic Antarctic silverfish *Pleuragramma antarcticum* (Goutte et al. 2013). D/T ratios were consistent with the sea ice conditions encountered: A year of reduced sea ice extent was characterized by low D/T ratios in top predators compared to a year of extended sea ice, supporting a lower contribution of ice-derived carbon in their diet (Goutte et al. 2013). While HBI biomarkers have been described in Arctic benthic macrofauna (Brown and Belt 2012a), the method has never been applied to Antarctic benthic species. Moreover, seasonal variations of HBI concentrations have been poorly investigated in response to ice melting.

In the present study, we focused on eight Antarctic fish species (108 specimens) collected throughout 2011. Fish fauna plays a key role in the Antarctic ecosystem, as they occupy most of the available ecological niches, including

pelagic, cryopelagic and benthic habitats (La Mesa et al. 2004a; Cherel et al. 2011). The first goal of this study was to compare HBI concentrations between two tissue types, namely the liver and white muscle. Our second aim was to describe HBI concentrations in coastal Antarctic fishes that are known to exploit different habitats. The third aim of the present study was to investigate a possible switch in food source from ice algae to pelagic phytoplankton during the year, by exploring seasonal changes in HBI concentrations.

## Materials and methods

### Study site and species

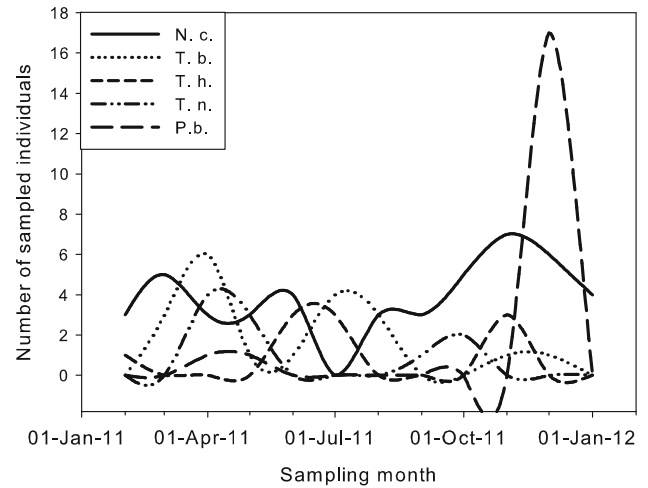
This study was carried out from February 21, 2011 to January 17, 2012 in the coastal waters of the Pointe Géologie Archipelago (Adélie Land, Antarctica,  $66^{\circ}40'S$ ,  $140^{\circ}01'E$ ), over a surface area of less than one square km. Eight species of Antarctic fishes ( $N = 108$ ) were caught underneath the sea ice and up to 87 m deep using different sampling methodologies (Table 1). For benthic species, we paid line out until we felt contact with the bottom and then reeled in just enough to keep our lure from hanging bottom. Each fish was identified to the species level based on morphological criteria, was measured (total length; nearest mm) and weighed (nearest g). Samples of white muscle (mean  $\pm$  SE:  $27.2 \pm 1.6$  g wet weight) and liver ( $1.5 \pm 0.2$  g wet weight) were excised and immediately frozen at  $-80$  °C until analysis in the laboratory. For 18 small individuals, the amount of liver sample was not sufficient for HBI analyses. Due to its small size, *P. antarcticum* (juvenile) was not dissected and HBI markers were analyzed from the whole body. Table 1 summarizes all the information concerning sampling and fish data. Sampling dates for each species are represented on Fig. 1.

### HBI analyses

Highly branched isoprenoids (HBIs) analyses were conducted as previously described in Goutte et al. (2013). Briefly, an internal standard (7-hexylnonadecane, 10  $\mu\text{L}$ , 0.02 mg/mL) was added to a subsample of freeze-dried biological tissue (around 1 g dry weight). After saponification (KOH, 4N, 2 h, 80 °C), the non-saponifiable lipids (NSL) were extracted into hexane ( $3 \times 10$  mL) and purified using open column chromatography (SiO<sub>2</sub> 50 g g<sup>-1</sup> NSL; hexane 8 mL). This hydrocarbon fraction was analyzed using an Agilent 7890A gas chromatograph (GC) fitted with a 30 m fused silica Agilent J&C GC column (0.25-mm i.d., 0.25- $\mu\text{m}$  film) coupled to an Agilent 5975C Series mass selective detector (MS) and with the Agilent Chemstation software. Diene and triene were identified by

**Table 1** Sample data, including scientific names of species, sample size of liver ( $N_{liver}$ ) and of muscle ( $N_{muscle}$ ), stage (Ad adult; Juv juvenile), sampling period (start and end, dd-mm-yy), habitat, total water depth (in m), sample collection depth, fishing gear, total length (TL, mean  $\pm$  SE, in mm) and mass (mean  $\pm$  SE, in g)

Species	N liver	N muscle	Stage	Sampling period		Habitat	Total water depth (m)	Sample collection depth	Sample collection	Fishing gear	TL (mm)	Mass (g)
				Start	End							
<i>Gymnodraco acuticeps</i>	1	1	Ad	28-11-11	28-11-11	Benthic	1	Underneath the sea ice	Trammel net	225	61	
<i>Pagothenia borchgrevinkii</i>	15	19	Ad	06-04-11	22-12-11	Cryopelagic	1	Underneath the sea ice	Fishing rod	138.47 $\pm$ 6.97	30.20 $\pm$ 6.66	
<i>Pleurogramma antarcticum</i>	0	1	Juv	28-11-11	28-11-11	Cryopelagic (juvenile)	1	Underneath the sea ice	Zooplankton net	42	-	
<i>Notothenia coriiceps</i>	41	46	Ad	21-02-11	17-01-12	Benthic	15–87	Seafloor	Fish trap, fishing rod	244.93 $\pm$ 6.06	205.44 $\pm$ 15.79	
<i>Trematomus bernacchii</i>	17	20	Ad	24-03-11	02-12-11	Benthic	5–38	Seafloor	Fish trap, fishing rod	188.50 $\pm$ 10.69	102.50 $\pm$ 14.99	
<i>Trematomus hansonii</i>	10	10	Ad	21-02-11	07-11-11	Benthic	38–87	Seafloor	Fish trap	230.86 $\pm$ 21.15	245.10 $\pm$ 40.45	
<i>Trematomus newnesi</i>	4	10	Ad	13-04-11	03-10-11	Semipelagic	5–15	Seafloor	Fishing rod	169.60 $\pm$ 7.57	50.00 $\pm$ 8.79	
<i>Trematomus pennellii</i>	1	1	Ad	21-02-11	21-02-11	Benthic	87	Seafloor	Fish trap	228	170	

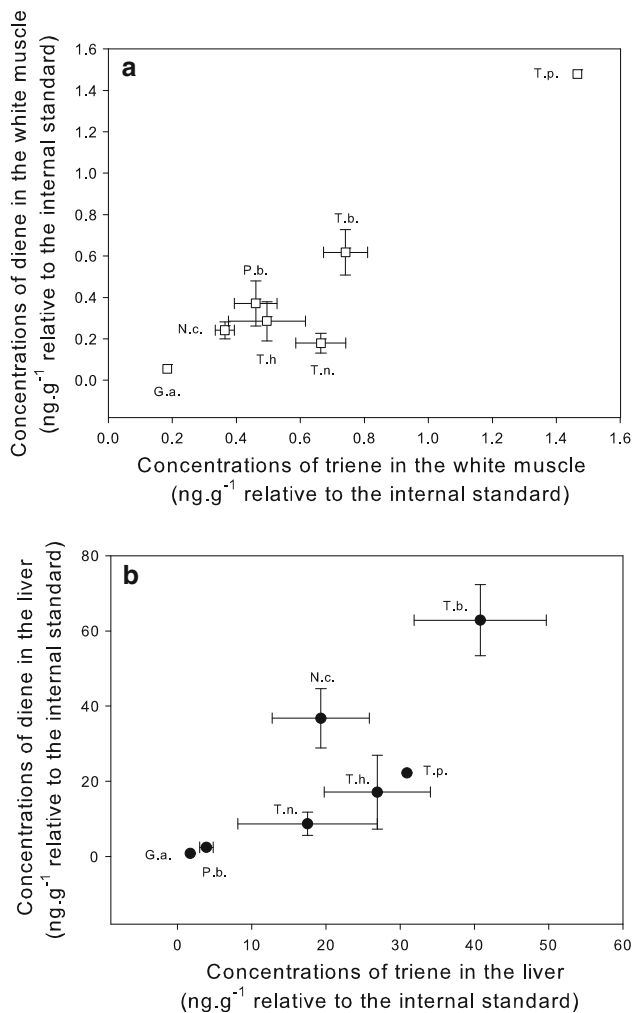


**Fig. 1** Number of sampled individuals per species according to sampling months (N. c.: *Notothenia coriiceps*, T. b.: *Trematomus bernacchii*, T. h.: *Trematomus hansonii*, T. n.: *Trematomus newnesi*, P. b.: *Pagothenia borchgrevinkii*)

comparing their GC retention indices and mass spectra with those of previously authenticated standards. Selective ion monitoring techniques were used to determine the abundances of diene ( $m/z$  348.3) and triene ( $m/z$  346.3) by peak area integration. HBI relative abundances were calculated based on their individual GC–MS responses and those of the internal standard (7-hexylnonadecane,  $m/z$  266). As we did not determine their individual response factors, diene and triene concentrations are thus expressed in  $ng\ g^{-1}$  dry weight relative to the internal standard. Procedural blanks were analyzed every 20 samples to ensure the absence of any contamination.

Statistical analyses

All statistical tests were performed using R 2.15.1 (R Development Team 2012). Firstly we compared the concentrations of diene, triene and the D/T ratio between the liver and the white muscle, by using generalized linear mixed models (GLMMs) with individual as a random effect. Then, we tested species differences in HBI concentrations and D/T ratio in the white muscle and in the liver separately, by using generalized linear models (GLMs) with gaussian family and an identity link function. *P. antarcticum*, *Gymnodraco acuticeps* and *Trematomus pennellii* were not included in the statistical tests, because only one specimen of each of them was collected. Multiple comparisons of means were conducted using Tukey’s contrasts. Finally, we investigated the seasonal variations of HBI markers in the white muscle and in the liver during the year, by performing generalized additive model (GAMs) with gaussian family and an identity link function to test a smooth effect of sampling date on HBI

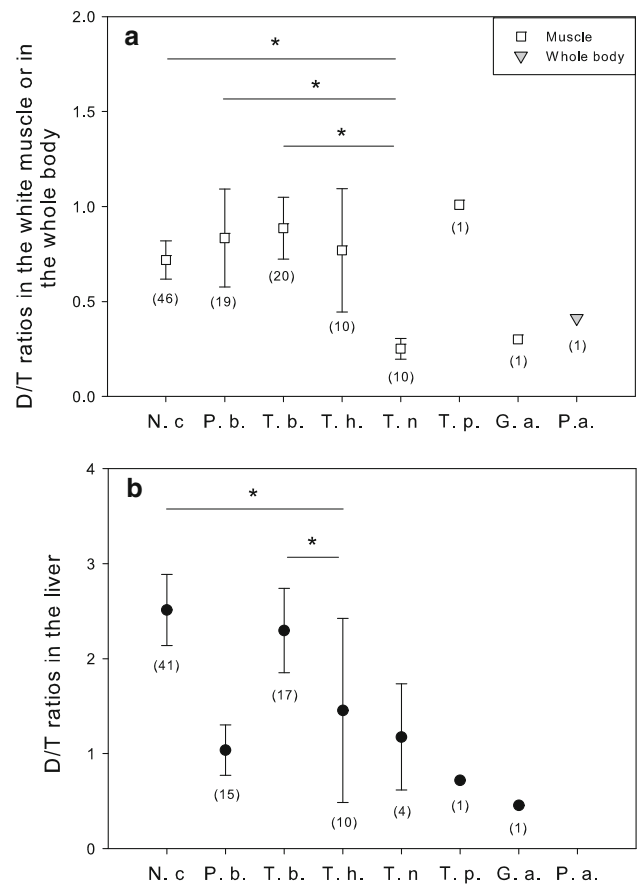


**Fig. 2** Diene and triene concentrations (mean and SE, in  $\text{ng g}^{-1}$  relative to the internal standard) measured in (a) white muscle and (b) liver of the eight Antarctic fish species (N. c.: *Notothenia coriiceps*, P. b.: *Pagothenia borchgrevinki*, T. b.: *Trematomus bernacchii*, T. h.: *Trematomus hansonii*, T. n.: *Trematomus newnesi*, T. p.: *Trematomus pennellii*, G. a.: *Gymnodraco acuticeps*, P. a.: *Pleuragramma antarcticum*)

concentrations, after taking into account species differences [D/T – species + s (sampling date)]. Because high sample sizes are required to use GAMs, the analyses were conducted on all species, except *P. antarcticum*, *G. acuticeps* and *T. pennellii*. Concentrations of diene and triene, as well as the D/T ratio, were log-transformed to meet modeling assumptions.

## Results

HBI concentrations were higher in the liver than in white muscle of the same individuals (diene:  $F_{1,86} = 59.067$ ,  $p < 0.001$ ; triene:  $F_{1,86} = 69.632$ ,  $p < 0.001$ ; D/T ratio:  $F_{1,86} = 20.637$ ,  $p < 0.001$ ).

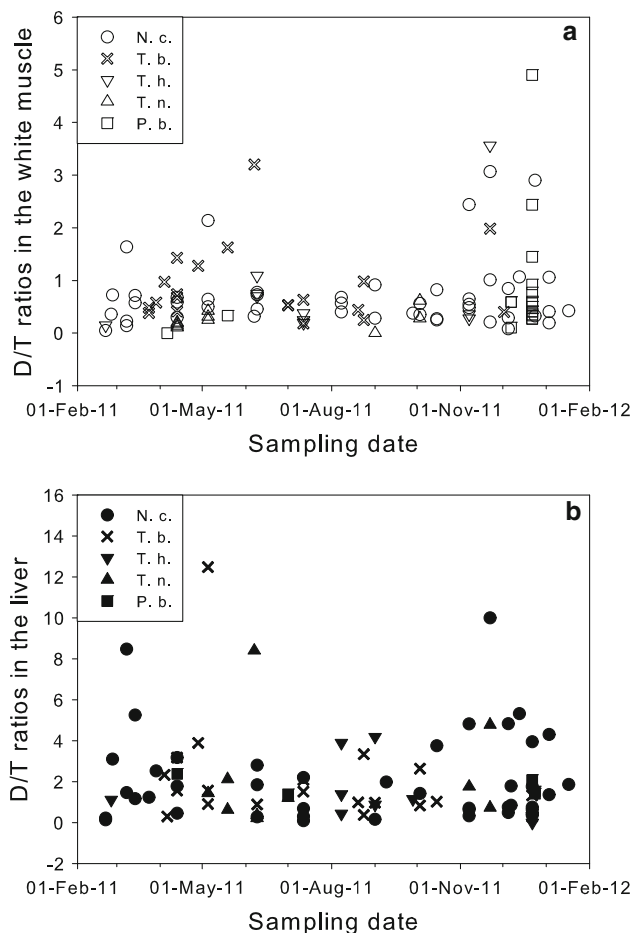


**Fig. 3** D/T ratios (mean and SE) in (a) white muscle or whole body and (b) in liver of the eight Antarctic fish species (N. c.: *Notothenia coriiceps*, P. b.: *Pagothenia borchgrevinki*, T. b.: *Trematomus bernacchii*, T. h.: *Trematomus hansonii*, T. n.: *Trematomus newnesi*, T. p.: *Trematomus pennellii*, G. a.: *Gymnodraco acuticeps*, P. a.: *Pleuragramma antarcticum*). The symbol “Asterisk” denotes significant differences between two fish species, using Tukey’s contrasts

## Inter-species differences in HBI markers

HBI concentrations measured in white muscle were significantly different among species (Figs. 2a, 3a, GLM, diene:  $F_{4,101} = 4.687$ ,  $p = 0.002$ ; triene:  $F_{4,101} = 5.733$ ,  $p < 0.001$ ; D/T ratio:  $F_{4,101} = 3.542$ ,  $p = 0.010$ ). Specifically, concentrations of diene were significantly higher in *T. bernacchii* compared to *Notothenia coriiceps* and *T. newnesi* ( $p < 0.035$  for Tukey’s contrasts), and concentrations of triene were significantly higher in *T. bernacchii* compared to *N. coriiceps* and *P. borchgrevinki*; they were also significantly higher in *T. newnesi* than in *N. coriiceps* ( $p < 0.043$  for Tukey’s contrasts). Muscle D/T ratios were significantly lower in *T. newnesi* compared to *N. coriiceps*, *T. bernacchii* and *P. borchgrevinki* ( $p < 0.018$  for Tukey’s contrasts).

Similarly, HBI concentrations measured in the liver were significantly different among species (Figs. 2b, 3b, diene:  $F_{4,83} = 19.583$ ,  $p < 0.001$ ; triene:  $F_{4,83} = 12.057$ ,



**Fig. 4** Seasonal variation in the D/T ratio measured in (a) white muscle or (b) liver of fish species (N. c.: *Notothenia coriiceps*, T. b.: *Trematomus bernacchii*, T. h.: *Trematomus hansonii*, T. n.: *Trematomus newnesi*, P. b.: *Pagothenia borchgrevinki*) from February 2011 to February 2012

$p < 0.001$ ; D/T ratio:  $F_{4,83} = 4.601$ ;  $p = 0.002$ ). Specifically, concentrations of diene were significantly higher in *T. bernacchii* compared to *N. coriiceps*, *P. borchgrevinki*, *T. newnesi* and *T. hansonii*; they were significantly lower in *P. borchgrevinki* compared to *N. coriiceps* and *T. hansonii* ( $p < 0.018$  for Tukey's contrasts). Concentrations of triene were significantly lower in *P. borchgrevinki* compared to *N. coriiceps*, *T. bernacchii* and *T. hansonii*; they were significantly higher in *T. bernacchii* compared to *N. coriiceps* ( $p < 0.004$  for Tukey's contrasts). Liver D/T ratios were significantly lower in *T. hansonii* compared to *N. coriiceps* and *T. bernacchii* ( $p < 0.015$  for Tukey's contrasts).

#### Seasonal variation in HBI concentrations

HBI concentrations in white muscle were related to sampling date in a nonlinear manner (GAMs, diene: edf = 6.209,  $F = 3.178$ ,  $p = 0.004$ ; triene: edf = 7.098,  $F = 2.191$ ,  $p = 0.034$ ; D/T ratio: edf = 4.675,  $F = 2.081$ ,

$p = 0.065$ ), after taking into account differences among species ( $p < 0.001$  for all tests). Specifically, a first peak of D/T ratio was observed from April to June and a second peak from the end of October to the end of December (Fig. 4a).

In contrast, HBI concentrations in the liver were not related to sampling date (GAMs, diene: edf = 1,  $F = 0.004$ ,  $p = 0.95$ ; triene: edf = 1,  $F = 0.105$ ,  $p = 0.746$ ; D/T ratio: edf = 1,  $F = 0.078$ ,  $p = 0.781$ , Fig. 4b), after taking into account differences among species ( $p < 0.001$  for all tests).

#### Discussion

The use of HBI markers in polar marine organisms is a recently developing approach, aiming at describing the relative contribution of carbon from ice algae versus pelagic phytoplankton in consumers (Brown and Belt 2012a; Brown et al. 2012, 2013a, b; Goutte et al. 2013). In a previous study, we showed that the D/T ratio was consistent with the relative contribution of ice algal-derived organic matter in the diet of Antarctic cryopelagic and pelagic consumers (Goutte et al. 2013). Here, we extended our investigation by including benthic and semipelagic fishes.

#### Species differences in HBI concentrations

HBI lipids were found in all specimens, confirming that sea ice and phytoplanktonic particulate organic matter are highly important as an indirect carbon source for fish species. Diene and triene were much more concentrated in the liver than in white muscle of the fish, as previously highlighted in seabirds (Goutte et al. 2013; Brown et al. 2013a). Little is known on the time-integrated assimilation rates and elimination rates of diene and triene within tissues. For other lipids, such as fatty acids, liver is considered to yield dietary information on few days to 1 week and muscle tissue about few weeks (Hobson 1993). The relative concentrations of diene and triene varied greatly among species, which could be attributed to species differences in incorporating, metabolizing and eliminating HBI compounds, as well as to differential feeding habits and trophic ecology.

*Pleuragramma antarcticum* is the predominant mid-water notothenioid fish in neritic Antarctic waters: It exploits open water habitats during adulthood, spawns underneath of the sea ice and spends its larval and juvenile stages within the cryopelagic communities (La Mesa et al. 2004a; La Mesa and Eastman 2012). The D/T ratio measured in juvenile *P. antarcticum* (0.41) was higher than those found in the adult stages (mean  $\pm$  SE:  $0.06 \pm 0.01$ , Goutte et al. 2013).

Although only one juvenile *P. antarcticum* was collected, this indicates that cryopelagic juveniles rely more heavily on ice-derived organic matter than the pelagic adults.

Secondly, the D/T ratios in white muscle were significantly higher in *P. borchgrevinki* than in *T. newnesi* in agreement with the feeding ecology of the two species: *P. borchgrevinki* relies extensively on sympagic amphipods and copepods (Hoshiai et al. 1989; La Mesa et al. 2004a), while *T. newnesi* is a semipelagic fish that feeds occasionally on cryopelagic items (Gon and Heemstra 1990; Vacchi and La Mesa 1995; Barrera-Oro and Piacentino 2007). This difference in D/T ratios was not found in the liver, probably due to the small number of analyses performed in this tissue (only four livers of *T. newnesi*).

Analysis of liver and muscle samples from *T. bernacchii* and *N. coriiceps* revealed high D/T ratio compared to the other fish species. It has been recently shown that organisms can accumulate IP<sub>25</sub> in their tissue during the Arctic winter when no sea ice particulate organic matter was being produced because they were feeding on benthic invertebrate prey (Brown et al. in press). In the shallow waters (<90 m depth) of the Ross Sea, *T. bernacchii* has been shown to rely on benthic amphipods and bivalves, such as the Antarctic scallop *Adamussium colbecki* (La Mesa et al. 2004b). These suspension feeders filter particulate organic matter and thus HBI lipids, and therefore, it is possible that the consumption of Antarctic scallops by *T. bernacchii* may explain the high concentrations of diene and triene observed in this species. The opportunistic and shallow benthic fish species *N. coriiceps* is known to feed both on algae and invertebrates (Hureau 1970). Large beds of macroalgae, as well as epiphytic and benthic diatoms, are thus consumed either directly by *N. coriiceps* (Iken et al. 2004), or indirectly through detrital pathways (Dunton 2001; Norkko et al. 2004). The contribution of these additional sources of organic matter in benthic fish species is not taken into account by the present HBI study. Although HBIs have been isolated in laboratory cultures of benthic diatoms from temperate areas (Johns et al. 1999; Belt et al. 2000; Rowland et al. 2001; Grossi et al. 2004), HBI productions by benthic or epiphytic diatoms have never been documented in Antarctica, raising one potential limitation for the use of HBIs to estimate the contribution of organic matter derived from benthic and/or epiphytic sources in the diet of coastal benthic fish. Further studies and validations are thus needed to better understand the potential of this recently developed method.

Large variances in the concentrations of HBI lipids were also observed among individual fishes of the same species. One possible reason could be attributed to the fact that these species are trophic generalists and opportunist feeders (Gon and Heemstra 1990; La Mesa et al. 2004b) and

that they modify their food sources and feeding habits during their life stages (Barrera-Oro and Piacentino 2007). Alternatively, HBI levels may vary according to sex and fish maturity. The presence of HBIs has been reported in eggs of benthic invertebrates and seabirds (Brown and Belt 2012a; Goutte et al. 2013), supporting the hypothesis of a decrease in maternal HBI levels after spawning.

#### Seasonal variation in HBIs concentrations

Blooms of sea ice algae differ considerably from those of the phytoplankton in terms of timing and distribution (Lizotte 2001). Investigating seasonal changes of D/T ratio in Antarctic fish species may shed some light on a switch in carbon source from ice algae to pelagic phytoplankton. We detected seasonal variations in the concentrations of diene, triene and the D/T ratios in white muscle, but not in the liver.

In white muscle, the D/T ratios tended to reach a first peak from April to June (fall–winter) and a second peak from end of October to end of December (spring), consistent with previous results on the chlorophyll a concentrations from sympagic diatoms in the Pointe Géologie Archipelago (Fiala et al. 2006). A first proliferation of sea ice communities has been shown to occur in the surface layer during the land-fast ice formation in April–May, whereas a second bloom was described in spring, just before the annual ice break up in November–December (Fiala et al. 2006). Blooms of Antarctic sympagic diatoms are associated with the production of diene (Massé et al. 2011), and the incorporation through the trophic web would explain the two seasonal peaks of D/T ratios in the white muscle of Antarctic fishes.

At last, it has been shown that a part of sea ice organic matters are not consumed immediately but are retained within the sediment (Mincks et al. 2005; Smith et al. 2006). This so-called sediment food bank is resuspended during the winter months, then being used by suspension feeders (Mincks et al. 2005; Smith et al. 2006). Increasing D/T ratios from April to June may therefore correspond to the direct and indirect use of sea ice particulate organic matter in the diet of Antarctic fish during winter months. In addition, macrobenthic organisms feed on or in the sediment all year round and are then preyed by benthic fish. Therefore, the fluctuations in D/T ratios in fish may be independent of the seasonal primary production.

Antarctic fish fauna plays a key role in the Antarctic marine ecosystem and is highly affected by sea ice condition. However, little is known about how changes in the timing of sea ice decay and formation will affect Antarctic fish communities. Although the present study pointed out some methodological considerations about the HBI approach, the use of HBIs has great potential for providing

novel information regarding species differences and seasonal changes in dietary sources within Antarctic fish species.

**Acknowledgments** We thank all the fieldworkers for sample collection and V. Klein for his excellent technical assistance in lipid purification. This work was funded by the European Research Council (ICEPROXY, Project Number 203441) and was logistically supported by the French Institute Paul-Emile Victor (IPEV Project Number 1010, ICHELIPIDS, G. Massé; 1124, REVOLTA, G. Lecointre). We thank Thomas Brown and two reviewers for their useful and constructive comments.

## References

- Arrigo KR, Thomas D (2004) Large scale importance of sea ice biology in the Southern Ocean. *Antarct Sci* 16:471–486
- Barrera-Oro E, Piacentino GLM (2007) Feeding habits of juvenile *Trematomus newnesi* (Pisces, Nototheniidae) at Potter Cove, South Shetland Islands, Antarctica. *Polar Biol* 30:789–796
- Belt ST, Allard WG, Massé G, Robert JM, Rowland SJ (2000) Important sedimentary sesterterpenoids from the diatom *Pleurosigma intermedium*. *Chem Commun* (6):501–502
- Belt ST, Allard WG, Massé G, Robert JM, Rowland SJ (2001a) Structural characterisation of C<sub>30</sub> highly branched isoprenoid alkenes (rhizenes) in the marine diatom *Rhizosolenia setigera*. *Tetrahedron Lett* 42:5583–5585
- Belt ST, Massé G, Allard WG, Robert JM, Rowland SJ (2001b) C<sub>25</sub> highly branched isoprenoid alkenes in planktonic diatoms of the *Pleurosigma* genus. *Org Geochem* 32:1271–1275
- Belt ST, Massé G, Allard WG, Robert JM, Rowland SJ (2001c) Identification of a C<sub>25</sub> highly branched isoprenoid triene in the freshwater diatom *Navicula sclesvicensis*. *Org Geochem* 32:1169–1172
- Belt ST, Massé G, Rowland SJ, Poulin M, Michel C, LeBlanc B (2007) A novel chemical fossil of palaeo sea ice: IP25. *Org Geochem* 38:16–27
- Brown TA, Belt ST (2012a) Identification of the sea ice diatom biomarker IP25 in Arctic benthic macrofauna: direct evidence for a sea ice diatom diet in Arctic heterotrophs. *Polar Biol* 35:131–137
- Brown TA, Belt ST (2012b) Closely linked sea ice–pelagic coupling in the Amundsen Gulf revealed by the sea ice diatom biomarker IP25. *J Plankton Res* 34:647–654
- Brown TA, Belt ST, Piepenburg D (2012) Evidence for a pan-Arctic sea-ice diatom diet in *Strongylocentrotus* spp. *Polar Biol* 35:1281–1287
- Brown TA, Bicknell AWJ, Votier SC, Belt ST (2013a) Novel molecular fingerprinting of marine avian diet provides a tool for gaining insights into feeding ecology. *Environ Chem Lett* 11:283–288. doi:10.1007/s10311-013-0402-x
- Brown TA, Belt ST, Ferguson SH, Yurkowski DJ, Davison NJ, Barnett JEF, Jepson PD (2013b) Identification of the sea ice diatom biomarker IP25 and related lipids in marine mammals: a potential method for investigating regional variations in dietary sources within higher trophic level marine systems. *J Exp Mar Biol Ecol* 441:99–104. doi:10.1016/j.jembe.2013.01.020
- Brown TA, Hegseth EN, Belt ST (in press) A biomarker-based investigation of the mid-winter ecosystem in Rijpfjorden, Svalbard. *Polar Biol*. doi:10.1007/s00300-013-1352-2
- Cherel Y, Koubbi P, Giraldo C, Penot F, Tavernier E, Moteki M, Ozouf-Costaz C, Causse R, Chartier A, Hosie G (2011) Isotopic niches of fishes in coastal, neritic and oceanic waters off Adélie land, Antarctica. *Polar Sci* 5:286–297
- Dunton KH (2001) d15 N and d13C measurements of Antarctic Peninsula fauna: trophic relationships and assimilation of benthic seaweeds. *Am Zool* 41:99–112
- Fiala M, Kuosa H, Kopczynska EE, Oriol L, Delille D (2006) Spatial and seasonal heterogeneity of sea ice microbial communities of first-year ice in the Terra Adélie area (Antarctica). *Aquatic Microb Ecol* 43:95–106
- Garrison DL (1991) Antarctic sea ice biota. *Am Zool* 31:17–33
- Gon O, Heemstra PC (1990) Fishes of the Southern Ocean. J.L.B Smith Institute of Ichthyology, Grahamstown
- Goutte A, Cherel Y, Houssais M-N, Klein V, Ozouf-Costaz C et al (2013) Diatom-specific highly branched isoprenoids as biomarkers in Antarctic consumers. *PLoS ONE* 8(2):e56504. doi:10.1371/journal.pone.0056504
- Grossi V, Beker B, Geenevasen JAJ, Schouten S, Raphael D, Fontaine M-F, Sinninghe Damsté JS (2004) C<sub>25</sub> highly branched isoprenoid alkene from the marine benthic diatom *Pleurosigma strigosum*. *Phytochem* 65:3049–3055
- Hobson KA (1993) Trophic relationships among high Arctic seabirds: insights from tissue-dependent stable-isotope models. *Mar Ecol Prog Ser* 95:7–18
- Hoshiai T, Tanimura A, Fukuchi M, Watanabe K (1989) Feeding by the Nototheniid fish, *Pagothenia borchgrevinki* on the ice-associated copepod, *Paralabidocera antarctica*. *Proc NIPR Symp Polar Biol* 2:61–64
- Hureau JC (1970) Biologie comparée de quelques Poissons antarctiques (Nototheniidae). *Bull Inst Océanogr Monaco* 68:1–244
- Iken K, Barrera-Oro ER, Quartino ML, Casaux RJ, Brey T (2004) Grazing by the Antarctic fish *Notothenia coriiceps*: evidence for selective feeding on macroalgae. *Antarct Sci* 9:386–391
- Johns L, Wraige EJ, Belt ST, Lewis CA, Massé G, Robert JM, Rowland SJ (1999) Identification of a C<sub>25</sub> highly branched isoprenoid (HBI) diene in Antarctic sediments, Antarctic sea-ice diatoms and cultured diatoms. *Org Geochem* 30:1471–1475
- La Mesa M, Eastman JT (2012) Antarctic silverfish: life strategies of a key species in the high-Antarctic ecosystem. *Fish Fish* 13:241–266
- La Mesa M, Eastman JT, Vacchi M (2004a) The role of nototheniid fish in the food web of the Ross Sea shelf waters: a review. *Polar Biol* 27:321–338
- La Mesa M, Dalù M, Vacchi M (2004b) Trophic ecology of the emerald notothen *Trematomus bernacchii* (Pisces, Nototheniidae) from Terra Nova Bay, Ross Sea, Antarctica. *Polar Biol* 27:721–728
- Lizotte MP (2001) The contributions of sea ice algae to Antarctic marine primary production. *Am Zool* 41:57–73
- Massé G, Belt ST, Crosta X, Schmidt S, Snape I et al (2011) Highly branched isoprenoids as proxies for variable sea ice conditions in the Southern Ocean. *Antarct Sci* 23:487–498
- Mincks SL, Smith CR, DeMaster DJ (2005) Persistence of labile organic matter and microbial biomass in Antarctic shelf sediments: evidence of a sediment ‘food bank’. *Mar Ecol Prog Ser* 300:3–19
- Nichols PD, Palmisano AC, Volkman JK, Smith GA, White DC (1988) Occurrence of an isoprenoid C<sub>25</sub> diunsaturated alkene and high neutral lipid content in Antarctic sea-ice diatom communities. *J Phycol* 24:90–96
- Norkko A, Thrush SF, Cummings VJ, Funnell GA, Schwarz AM, Andrew NL, Hawes I (2004) Ecological role of *Phyllophora antarctica* drift accumulations in coastal soft-sediment communities of McMurdo Sound, Antarctica. *Polar Biol* 27:482–494
- R Development Team 2.15.1 (2012-06-22) The R foundation for statistical computing

- Rowland SJ, Belt ST, Wraige EJ, Masse G, Roussakis C, Robert JM (2001) Effects of temperature on polyunsaturation in cytosolic lipids of *Haslea ostrearia*. *Phytochem* 56:597–602
- Sinninghe Damsté JS, Schouten S, Rijpstra WIC, Hopmans EC, Peletier H, Gieskes WWC, Geenevasen JAJ (1999) Structural identification of the C<sub>25</sub> highly branched isoprenoid pentaene in the marine diatom *Rhizosolenia setigera*. *Org Geochem* 30:1581–1583
- Sinninghe Damsté S, Rijpstra WIP, Coolen MJL, Schouten S, Volkman JK (2007) Rapid sulphurisation of highly branched isoprenoid (HBI) alkenes in sulphidic Holocene sediments from Ellis Fjord, Antarctica. *Org Geochem* 38:128–139
- Smith CR, Mincks S, DeMaster DJ (2006) A synthesis of benthopelagic coupling on the Antarctic shelf: food banks, ecosystem inertia and global climate change. *Deep Sea Res Part II* 53:875–894
- Vacchi M, La Mesa M (1995) The diet of the Antarctic fish *Trematomus newnesi* Boulenger, 1902 (Nototheniidae) from Terra Nova Bay, Ross Sea. *Antarct Sci* 7:37–38
- Volkman JK, Barrett SM, Dunstan GA (1994) C<sub>25</sub> and C<sub>30</sub> highly branched isoprenoid alkenes in laboratory cultures of two marine diatoms. *Org Geochem* 21:407