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1 **Biomarker-based H-Print quantifies the composition of mixed sympagic and**  
2 **pelagic algae consumed by *Artemia* sp.**

3

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13

14 Abstract

15

16 Quantifying the importance of sea ice microalgae as a food source in Arctic ecosystems is  
17 becoming an increasingly important research objective as sea ice extent and thickness  
18 continue to reduce. Recently, the analysis of certain diatom-derived highly branched  
19 isoprenoid (HBI) lipid biomarkers has provided a means of qualitatively distinguishing  
20 between sympagic (sea ice) and pelagic algae in Arctic animals. By combining the  
21 abundances of these lipids into an HBI-fingerprint, or “H-Print”, an estimate of the relative  
22 proportions of HBIs from each algal source can also be made. Although H-Print analysis of  
23 animal tissues in the Arctic is starting to provide such information, it has not yet been  
24 established to what extent H-Prints in animals provide a true reflection of the content of their  
25 algal food source. Here it is demonstrated that the H-Print determination can yield reliable

26 estimates of mixed sympagic/pelagic algal content both within a food source of known  
27 composition, and in a primary consumer fed on it. In doing so, the utility of the H-Print is  
28 extended towards providing quantitative estimates of the relative importance of sympagic  
29 algae to animal diet. To achieve this, a series of 5 algal samples were prepared with known  
30 %sympagic algal content ranging from 0 to 100%. Analysis of these samples led to a  
31 comparison of different regression models based upon H-Prints using 4 different  
32 combinations of individual HBIs. A linear model comprising 3 HBIs (2 sympagic and 1  
33 pelagic) provided the most accurate estimates of the sympagic content (-1%, 50% and 101%)  
34 for samples containing 0%, 50% and 100% sympagic algae. This linear model was then used  
35 to estimate the proportion of sympagic algae in *Artemia* sp. (-3%, 44% and 101%) fed on the  
36 same algal mixtures in the laboratory. The similarity between H-Prints in mixed algae and  
37 *Artemia* sp. suggested that H-Prints were not altered substantially by grazing, and this was  
38 also confirmed by analysis of the remaining water (containing ungrazed algae and faecal  
39 pellets), where H-Prints were not significantly different from those obtained for the algae or  
40 *Artemia* sp. ( $p = >0.3$ ). This study has extended the usefulness of the H-Print as an approach  
41 to determine ecosystem change in the future. Indeed, the ability of the H-Print to provide  
42 quantitative estimates of the importance of sympagic algae to Arctic animals is likely to result  
43 in valuable data that can be used for modelling the broader ecological impact of reducing sea  
44 ice extent.

45

46 Keywords: Highly branched isoprenoid (HBI), H-Print, sea ice algae, sympagic carbon,  
47 foodweb, Arctic

48

49

50

51 1 Introduction

52

53 Arctic sea ice provides a habitat for ice-adapted microalgae (Dieckmann and Hellmer, 2010),  
54 which, in turn, provide food for a wide range of heterotrophic organisms (Arrigo, 2014). With  
55 Arctic sea ice extent reducing (Meier et al., 2014; Stroeve et al., 2012), there is a need to  
56 understand the impact of an associated reduction in sea ice microalgae as they provide an  
57 important energy source for the ecosystem (Arrigo, 2014). Fundamental to this understanding  
58 is being able to ascertain, quantitatively, the sympagic and pelagic components of an animal's  
59 diet. In order for this to be achieved, however, it is first necessary to be able to identify  
60 methods by which sympagic and pelagic algae can be both distinguished and quantified. Such  
61 methods may, potentially, be available through the identification of signature chemical  
62 tracers of each algal type.

63

64 Highly branched isoprenoid (HBI) lipids are secondary metabolites that have been reported in  
65 a number of marine diatom genera that regularly form components of both sympagic and  
66 pelagic algae. For instance, in the Arctic, the sea ice diatoms *Haslea crucigeroides*, *H.*  
67 *spicula*, *H. kjellmanii* and *Pleurosigma stuxbergii* var *rhomboides* biosynthesise certain HBIs  
68 (e.g. I and III; Fig. 1.; Brown et al., 2014c) that have a distinctive carbon isotopic  
69 composition when found in the environment ( $\delta^{13}\text{C} = -19\pm 2\text{‰}$  and  $-18\pm 1\text{‰}$ ; Belt et al., 2008).  
70 While these sympagic HBIs are regularly reported in samples of Arctic sea ice (Belt et al.,  
71 2007, 2013; Brown et al., 2011), surface (Belt and Müller, 2013; Navarro-Rodriguez et al.,  
72 2013; Smik et al., 2016; Stoyanova et al., 2013; Xiao et al., 2015) and downcore (Belt et al.,  
73 2015; Müller et al., 2009, 2012; Polyak et al., 2016; Stein et al., 2016; Vare et al., 2009)  
74 sediments, as yet, they have not been found in pelagic water samples from ice-free locations.  
75 In contrast, other HBIs (including, II, IV, V, VI, VII and VIII; Fig. 1) are frequently reported

76 within pelagic water samples from sub-polar and temperate regions (Belt, et al. 2000; Cooke  
77 et al., 1998; Dunlop and Jefferies, 1985; He et al., 2016; Kaiser et al., 2016; Xu et al., 2006),  
78 and represent common components of marine sediments worldwide (Belt et al., 2000). These  
79 pelagic HBIs have been shown to be biosynthesised by temperate diatoms including  
80 *Berkeleya rutilans* (Brown et al., 2014b), *H. ostrearia* (Volkman et al., 1994; Wraige et al.,  
81 1997), *Pseudosolenia calcar-avis* (Kaiser et al., 2016), *Pleurosigma intermedium* (Belt et al.,  
82 2000; Brown and Belt, 2016), *P. strigosum* (Grossi et al., 2004) and *Rhizosolenia setigera*  
83 (Rowland et al., 2001; Volkman et al., 1994), making them ideal indicators of pelagic algae.  
84 The HBI biomarker-based H-Print (Brown et al., 2014d) provides a means of numerically  
85 combining all of these HBI biomarker abundances into a single index, thereby providing a  
86 measure of the relative composition of sympagic and pelagic algae in a sample of mixed  
87 content. While end-member H-Print values from sympagic and pelagic algae are relatively  
88 straightforward to interpret (Brown et al., 2014a,d), as yet, it has not been established  
89 whether intermediate values of the H-Print accurately reflect the relative proportions of  
90 mixed sympagic/pelagic algae composition.

91  
92 The main aim of the current study, therefore, was to identify a numerical model that  
93 described the relationship between the H-Print and the proportion of sympagic and pelagic  
94 algae for samples created in the laboratory. To achieve this, HBIs were first quantified in a  
95 series of samples made by combining varying proportions of both sympagic and pelagic  
96 algae. The abundances of various HBI biomarkers were then combined to produce H-Print  
97 values, which were subsequently used to establish a regression model. Further, in order to test  
98 the applicability of the H-Print approach within an animal grazing context, H-Prints derived  
99 from analysis of algal food sources of known composition were compared with those  
100 obtained from *Artemia* sp. fed on the same samples.

102 2 Material and methods

103

104 2.1 Diatom composition of sympagic and pelagic algae samples

105

106 For the determination of H-Print values in sympagic algae, samples of floating sea ice algae  
107 aggregates were used that had been collected from Resolute Bay, Nunavut, Canada (Brown et  
108 al., 2014c). Aggregates contained the HBI biosynthesising sea ice diatoms *H. crucigeroides*,  
109 *H. spicula*, *H. kjellmanii*, *P. stuxbergii* var *rhomboides* (combined total ca. 2%), with the  
110 remainder comprising mainly (ca. 80%) *Navicula pelagica*, *Pauliella taeniata* and *Nitzschia*  
111 *frigida* (Brown et al., 2014c). Samples to represent pelagic algae were prepared by combining  
112 cultures of known temperate HBI biosynthesising diatom species (*P. intermedium* (ca. 1%),  
113 *P. planktonicum* (ca. 0.5%), *P. sp.* (ca. 1%) and *H. ostrearia* (ca. 2%)) with a non-HBI  
114 producing centric diatom (*Thalassiosira weissfloggi*; ca. 95%). Total diatom cell abundances  
115 in 10 mg subsamples were comparable for both sympagic ( $3.1 \times 10^6$  cell L<sup>-1</sup>) and pelagic ( $3.5$   
116  $\times 10^6$  cell L<sup>-1</sup>) algae.

117

118 2.2 Rearing and feeding of *Artemia* sp. - experimental setup

119

120 For feeding experiments, *Artemia* sp. were chosen since the *Artemiidae* are considered to be  
121 continuous, non-selective filter feeders (Evjemo and Olsen, 1999), reaching saturated  
122 ingestion capacity between  $2 \times 10^3$  and  $1 \times 10^4$  cells L<sup>-1</sup> (da Costa et al., 2005; Reeve, 1963),  
123 which is below the cell abundances used here. About 1000 decapsulated *Artemia* sp. eggs  
124 (Waterlife Research ind. Ltd.) were rinsed with deionised water (ca. 10 mL), transferred to  
125 1.5 L clear plastic flasks (Corning) containing artificial seawater with a salinity of 32 (1 L  
126 milli-Q water; 32 g TropicMarin® salt), and maintained in suspension by aeration. During

127 rearing, light intensity was maintained below  $1 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ . From 3 days after  
128 hatching, a regime of 100% water changes, immediately followed by feeding (ca. 0.5 g  
129  $\text{H}^2\text{Ocean}^{\text{Pro+}}$ ), was carried out every 2–3 days. At >40 days old, 20 individual *Artemia* sp.  
130 were selected randomly from the rearing stock and transferred, along with 10 mg algae, to  
131 experimental flasks containing artificial seawater. Experimental flasks were maintained at  
132  $20 \pm 2.5^\circ\text{C}$  and ca.  $2 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ , with algae sustained in suspension by vigorous  
133 aeration. After feeding on algae for 24 h, *Artemia* sp. were removed from the water using a  
134 plastic pipette and pooled ( $n=20$ ) for analysis of HBI content. The remaining water  
135 containing ungrazed algae and faecal pellets was filtered (Whatman GF/F) and analysed for  
136 HBI content.

137

### 138 2.3 Extraction and analysis of HBI lipids

139

140 Extraction of HBI lipids was carried out on algal samples, *Artemia* sp. and filtered water as  
141 described previously (Brown et al., 2014a), and the subsequent analysis of purified non-polar  
142 extracts containing HBIs was carried out using gas chromatography–mass spectrometry (GC–  
143 MS) techniques according to Belt et al. (2012).

144

### 145 2.4 Calculation of the HBI biomarker H-Print

146

147 HBI abundances were obtained from GC–MS selective ion monitoring (SIM) output (see Belt  
148 et al., 2012) using the mass spectral intensities of the molecular ion for each HBI (Brown et  
149 al., 2014d). Individual HBI abundances were normalised according to totals derived from all  
150 HBIs. The resulting distribution provided the basis for the H-Print (Brown et al., 2014d),  
151 which is defined, here, as the proportion of HBIs from pelagic diatoms relative to those from

152 both sympagic and pelagic diatoms to provide H-Print values within the range 0–100% (Eq.  
153 1).

154

155 Eq. 1.

$$156 \quad H - \text{Print} (\%) = \frac{(\text{pelagic HBIs})}{(\text{sympgaic HBIs} + \text{pelagic HBIs})} \times 100$$

157

158 To represent pelagic algae, 4 combinations of HBIs were tested. Firstly, for H-Print<sup>1</sup>, all HBI  
159 isomers, for which the chemical structures and at least one biological source organism were  
160 also known (Eq. 2), were used. For H-Print<sup>2</sup> (Eq. 3), only V and VI were used, since these are  
161 the most common HBIs in phytoplankton (Belt et al., 2000). H-Print<sup>3-4</sup> used only V, as it was  
162 absent from the sympagic algae (Eq. 4). To represent biomarkers of sympagic algae, I and III  
163 were chosen for H-Prints<sup>1-3</sup> (Eq. 2–4) since these are the only HBIs in this study that are  
164 known to have a sea ice diatom source (Brown et al., 2014c). For H-Print<sup>4</sup> (Eq. 5) only I was  
165 included as a sympagic component since it was not present in the pelagic algae.

166 Eq. 2.

$$167 \quad H - \text{Print}^1 (\%) = \frac{(\text{II} + \text{IV} + \text{V} + \text{VI} + \text{VII} + \text{VIII})}{(\text{I} + \text{II} + \text{III} + \text{IV} + \text{V} + \text{VI} + \text{VII} + \text{VIII})} \times 100$$

168 Eq. 3.

$$169 \quad H - \text{Print}^2 (\%) = \frac{(\text{V} + \text{VI})}{(\text{I} + \text{III} + \text{V} + \text{VI})} \times 100$$

170 Eq. 4.

$$171 \quad H - \text{Print}^3 (\%) = \frac{(\text{V})}{(\text{I} + \text{III} + \text{V})} \times 100$$

172 Eq. 5

$$173 \quad H - \text{Print}^4 (\%) = \frac{(\text{V})}{(\text{I} + \text{V})} \times 100$$



174

175 2.5 Statistical design

176

177 All experiments were carried out in replicate ( $n=5$ ) and, where *Artemia* sp. were involved,  
178 each replicate contained 20 randomly selected individuals, resulting in 100 *Artemia* sp. being  
179 used for each treatment. Statistical analysis was performed in R Studio v0.99.441 (R-Core-  
180 Team, 2016). ANOVA was used to compare the H-Prints of algae, *Artemia* sp. and filtered  
181 water for each treatment. Least squares regression fits were used for comparison of the  
182 different H-Print equations across all treatments. Regression models were evaluated on the  
183 basis of their ability to predict known algal composition. Predictions of the sympagic algae  
184 content of samples based on H-Prints was done using the ‘predict()’ function in base R with  
185 confidence intervals of 99%.

186

187 4 Results

188

189 4.1 Quantification of HBIs and H-Prints in algae

190

191 Samples of sympagic algae were dominated by HBIs I and III, which comprised  $36\pm 5\%$  and  
192  $44\pm 5\%$  of total HBIs, respectively (Fig. 2). The remaining HBIs were, individually, all less  
193 than 10%, while V and VIII were absent. In contrast, the most abundant HBI in the pelagic  
194 algae was V ( $33\pm 5\%$ ), with VI and VII contributing  $15\pm 3\%$  and  $20\pm 2\%$ , respectively. Other  
195 HBIs were each less than 6%, while I and IV were absent from pelagic algae (Fig. 2). For  
196 samples consisting exclusively of sympagic algae, calculations using Eq. 2–5 resulted in H-  
197 Prints varying between 0–25%, with Eq. 4 and Eq. 5 both giving H-Prints of  $0\pm 0\%$ , while Eq.  
198 2 gave the highest values ( $18\pm 7\%$ ). For pelagic algae samples, the variability in H-Print  
199 values was much less (97–100%), with Eq. 4 and Eq. 5 providing the lowest ( $97\pm 1\%$ ) and  
200 highest ( $100\pm 0\%$ ) values, respectively. For composite samples containing both sympagic and  
201 pelagic algae, H-Prints showed more variability than for end-member algae samples. For  
202 example, H-Prints from composite samples containing 50% sympagic algae ranged from 42  
203 to 80% with, on average, Eq. 4 giving the lowest ( $53\pm 8\%$ ) values. Highest values were  
204 obtained using Eq. 5 ( $73\pm 7\%$ ).

205

206 Regression models derived from Eq. 2, 3 and 5 provided estimates of 109–129% sympagic  
207 algae for the sympagic end-member sample (Fig. 3, Table 1). The predictive model derived  
208 from Eq. 4 estimated the sympagic component to be 101%. For the 50% sympagic algae  
209 sample, the models derived from Eq. 2, 3 and 5 gave estimates above the known sympagic  
210 contribution (59%, 67% and 61%, respectively), while Eq. 4 predicted 50%. For pelagic end-  
211 member samples containing 0% sympagic algae, models using Eq. 2, 3 and 4 all yielded low

212 H-Prints (4%, 6% and -1% sympagic algae, respectively), while prediction from the model  
213 derived using Eq. 5 estimated a 13% contribution from sympagic algae, even though it was  
214 absent.

215

216 4.2 H-Prints from analysis of *Artemia* sp. and filtered water samples

217

218 H-Print data, calculated using Eq. 4, for *Artemia* sp. and filtered water samples were not  
219 significantly different from those obtained from algae for any of the sympagic/pelagic  
220 compositions used ( $p = >0.3$ ). Accordingly, almost all of the H-Prints calculated for *Artemia*  
221 sp. and filtered water fell well within the 99% confidence interval attached to the model  
222 derived from mixed algae (Fig. 4a and 4b).

223

224 5. Discussion

225

226 5.1 Selection of HBIs for use in the H-Print

227

228 In order to make comparisons of food source (as defined by the H-Print) in grazers more  
229 achievable, it was necessary to first identify the model that gave the most accurate  
230 relationship between H-Prints and the known algal composition. This selection focused on  
231 two main criteria; 1) accuracy of estimates and 2) confidence of the model. The most accurate  
232 model was derived from H-Print<sup>3</sup> using I, III and V as the constituent HBIs, which estimated  
233 the sympagic and pelagic end-members at 101% and -1%, respectively (Table 1). In contrast,  
234 models derived from the other H-Prints (1, 2 and 4) overestimated the sympagic component  
235 at >109% and >4% for the sympagic and pelagic end-members, respectively. In assessing the  
236 confidence intervals of the models, it was also found that the model using H-Print<sup>3</sup> had the  
237 smallest mean confidence interval range (29; Table 1), indicating the least uncertainty of all  
238 the models, while the remaining models had mean confidence interval ranges >37. The linear  
239 model derived from H-Print<sup>3</sup> was therefore used to predict sympagic/pelagic algae  
240 composition from hereon.

241

242 5.2 Comparison of H-Prints in algae, filtered water and *Artemia* sp.

243

244 Having demonstrated that the H-Print could provide reliable estimates of the  
245 sympagic/pelagic proportion of mixed algal samples of known composition, it was then  
246 necessary to test whether the H-Print could also provide reasonable estimates of the  
247 sympagic/pelagic composition of algal food consumed by animals. To be successful,  
248 zooplankton needed to feed, non-selectively, upon each algal treatment without alteration of

249 the H-Print. During the feeding experiments, there were no mortalities of *Artemia* sp., and the  
250 consistent, rapid appearance of visible faecal pellets indicated that non-selective grazing  
251 occurred for all experimental treatments. Experiments were run over 24 h to give *Artemia* sp.  
252 suitable opportunity to consume algae, and it is estimated that *Artemia* sp. consumed ca.  
253 150% of the available algae during each experiment. This was determined by comparing the  
254 amount of HBI I in the 10 mg sympagic algae supplied to *Artemia* sp. (ca. 0.59  $\mu\text{g}$ ), with that  
255 in pooled *Artemia* sp. after 24 h (ca. 0.02  $\mu\text{g}$ ), which indicated that ca. 3% of the algae was  
256 present in the guts of *Artemia* sp. Based on an assumption of a gut passage time of 20–30  
257 minutes (Nimura, 1989), it is estimated that ca. 50 gut passages per individual (ca. 1000 for  
258 20 individuals) occurred over the duration of each experiment, potentially resulting in 15 mg  
259 (150%) algae being consumed. This outcome is supported by the experiments of Reeve  
260 (1963) who showed that *Artemia* sp. consumed *Phaeodactylum tricornutum* at ca.  $4 \times 10^5$  cells  
261  $\text{h}^{-1}$ , which, over 24 h, corresponds to ca. 300% of our  $3.1 \times 10^6$   $\text{cell L}^{-1}$ , and seems quite  
262 feasible given the tendency for coprophagy in captive zooplankton (Werner, 2000). Despite  
263 the efficient grazing of sympagic algae in the current experiments, *Artemia* sp. did not alter  
264 the relative distributions of individual HBI biomarkers. Indeed, the majority (80%) of mean  
265 H-Prints derived from water and *Artemia* sp. fell within the 99% confidence interval of the  
266 regression model calculated using the algal H-Print<sup>3</sup> (Fig. 4b), suggesting that *Artemia* sp.  
267 grazed non-selectively on all treatments, without alteration of the H-Print. It is concluded,  
268 therefore, that the regression model, based upon algal H-Prints, remains accurate for *Artemia*  
269 sp., permitting application of the regression model to predict the sympagic/pelagic proportion  
270 of zooplankton diet.

271

272 5.3 Environmental application of the H-Print

273

274 Although it has been shown here that the H-Print approach can yield reliable estimates of the  
275 sympagic/pelagic composition of algae in the laboratory, even after being consumed by  
276 zooplankton, it is probably also important to consider some additional factors that may be  
277 important when using this method in environmental settings in the future. Three such factors  
278 are considered briefly here. Firstly, HBIs should be readily detectable in the environment.  
279 Recently, the ubiquity of HBIs has been established following the widespread reporting of  
280 these lipids in, for example, sea ice (Belt et al., 2007,2013,2016; Brown et al., 2011; Massé et  
281 al., 2011; Nichols et al., 1988), sediments (Belt and Müller, 2013; Navarro-Rodriguez et al.,  
282 2013; Xiao et al., 2015), zooplankton (Brown and Belt, 2012; Cripps, 1995), fish (Brown et  
283 al., 2015; Goutte et al., 2014b), birds (Brown et al., 2013a,2015) and marine mammals  
284 (Brown, et al., 2013a,2014a; Goutte et al., 2014a) from Arctic, Antarctic and temperate  
285 environments. Second, it is suggested that identification of the H-Print formula that best  
286 reflects the composition of natural sympagic and pelagic algae may also be important. In the  
287 current study, it was shown that a key factor in identifying such a formula was selection of  
288 HBIs that provide a balanced contribution from sympagic and pelagic sources. Achieving this  
289 balance is, to some extent, simplified by the shared ability of sympagic and pelagic algae to  
290 biosynthesise HBIs, thereby minimising any potential complications associated with  
291 comparing lipids from different sources; a problem frequently associated with more common  
292 lipids such as fatty acids or sterols which can have a wide range of sources (e.g. Natalia et al.,  
293 1999; Volkman et al., 1986,1998), including, in some cases, animals themselves.  
294 Nonetheless, the proportion of sympagic/pelagic HBIs will be dependent upon the overall  
295 abundances of HBI-biosynthesising diatoms present. On this basis, it is anticipated that H-  
296 Print<sup>3</sup> may, as it did in the experiments described herein, provide the most realistic estimates  
297 for environmental samples, since the species known to produce I, III and V have similar  
298 abundances in the environment. For example, the diatoms that produce I and III have a

299 consistent abundance in sea ice throughout the Arctic (1–5%; Brown et al., 2014c and  
300 references therein), while *Pleurosigma* spp. and *Rhizosolenia* spp. (known sources of V) are  
301 also typically 1–5% of species in pelagic diatom assemblages (Mather et al., 2010; von  
302 Quillfeldt, 2000). On the other hand, if the abundances of the sympagic or pelagic sources  
303 should differ substantially from this, it is possible that H-Print<sup>3</sup> may not be the best predictor  
304 of algal composition and a different combination of HBIs may give more reliable estimates.  
305 In order to obtain the most reliable estimates of algal composition in samples collected from  
306 the environment, it is therefore recommended that H-Prints for both sympagic and pelagic  
307 algae are determined on a case-by-case basis using samples collected from the environment  
308 being studied. Accordingly, the appropriate model can thus be selected that best estimates the  
309 composition of mixed algae samples.

310

311 Finally, differential modification of HBI lipid distributions within animals would likely result  
312 in inaccurate estimates of algae composition, regardless of which H-Print formula is used. As  
313 such, it is important that, once consumed by animals, HBIs do not become modified from  
314 their original source distributions, as was the case for *Artemia* sp. described herein. In the  
315 Arctic, the most abundant zooplankton are usually *Calanus* spp. (Auel and Hagen, 2002;  
316 Søreide et al., 2008) and, in contrast to *Artemia* sp., these do accumulate lipids (Graeve et al.,  
317 2005; Pond and Tarling, 2011). The close similarity of *Calanus* spp. H-Prints with those from  
318 pelagic algae (Brown et al., 2014d), however, in addition to those between some higher  
319 trophic level organisms and sympagic algae (Brown et al., 2014d), suggest that HBI  
320 distributions are not noticeably impacted by animals. Indeed, quantitative assessment of the  
321 relative proportions of I and III in over 300 ringed seals showed a consistent ratio (1:2.7) that  
322 aligned closely to typical values from sea ice (e.g. 1:2.1 (Belt et al., 2013) and 1:2.9 (Brown,  
323 2011)), further supporting the finding here that H-Prints are unlikely to be significantly

324 altered by animals. Confirmation of this, however, will likely require further analyses of  
325 larger sample sets that focus on Arctic animals feeding on prey with known H-Print  
326 signatures.

327

328 In summary, the data presented herein demonstrate that the biomarker-based H-Print has the  
329 potential to provide reliable, quantitative predictions of the sympagic/pelagic composition of  
330 animal diet. This conclusion is based upon a series of controlled laboratory experiments from  
331 which, first, a linear regression model was created, where H-print values reflected the relative  
332 proportions of sympagic and pelagic algae in mixtures of known composition. In addition,  
333 there was no significant difference in the numerical values of H-Prints measured in these  
334 samples of mixed algae and in zooplankton that had been fed these food sources. The  
335 potential for the H-Print method to provide quantitative estimates of the role that sympagic  
336 algae play in animal diet will likely lead to valuable new field data for modelling the broader  
337 ecological impacts of reducing Arctic sea ice extent.

338

339



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523 Table 1. Estimates of the sympagic contribution to mixed sympagic/pelagic algae samples

	<i>H-Print</i> <i>Eq.#</i>	<i>R</i> <sup>2</sup>	<i>Residual</i> <i>(se)</i>	<i>df</i>	<i>p</i>	Model estimates of sympagic contribution (95% CI) to composite algae			Mean CI range
						0:100	50:50	100:0	
<b>Composition</b> <b>(S:P)</b>									
<b>H-Print<sup>1</sup></b>	2	0.95	8.6	23	<0.01	4 (-14 – 23)	59 (41 – 77)	113 (95 – 133)	37
<b>H-Print<sup>2</sup></b>	3	0.92	10.4	23	<0.01	6 (-16 – 29)	67 (45 – 89)	129 (104 – 153)	46
<b>H-Print<sup>3</sup></b>	4	0.97	6.6	23	<0.01	-1 (-16 – 14)	50 (36 – 64)	101 (87 – 116)	29
<b>H-Print<sup>4</sup></b>	5	0.89	11.7	23	<0.01	13 (-11 – 39)	61 (37 – 86)	109 (83 – 135)	50

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526 Figure legends

527 Figure 1. Structures of diatom highly branched isoprenoid (HBI) lipid biomarkers described  
528 in this study.

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530 Figure 2. Relative proportions of highly branched isoprenoids (HBIs) in sympagic and  
531 pelagic algae used in this study.

532

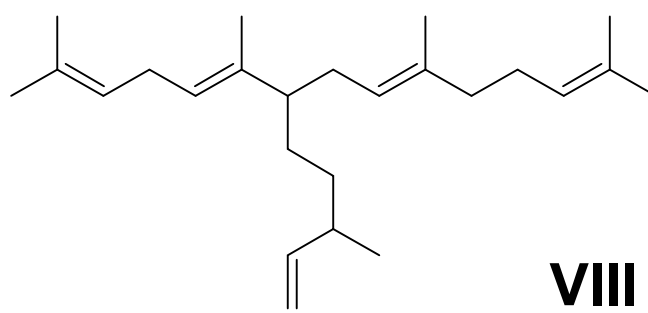
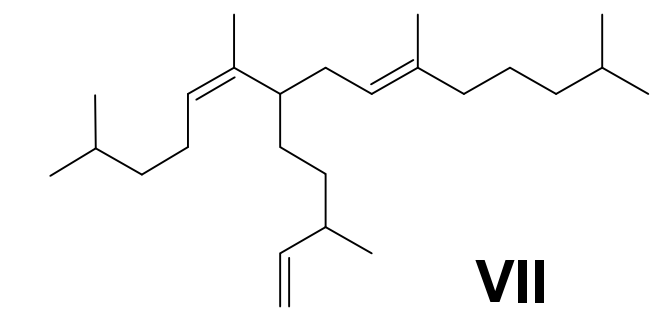
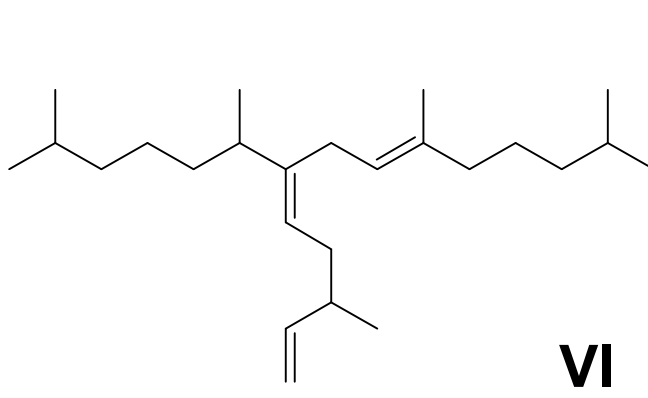
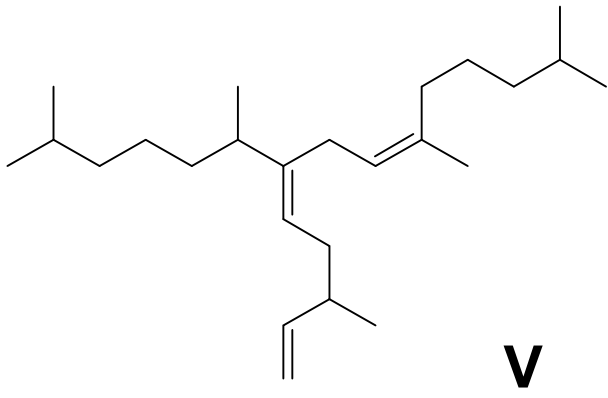
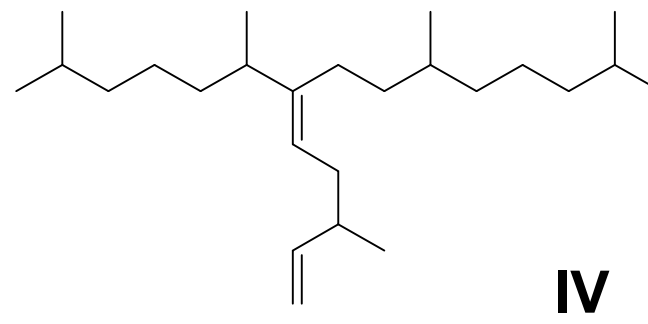
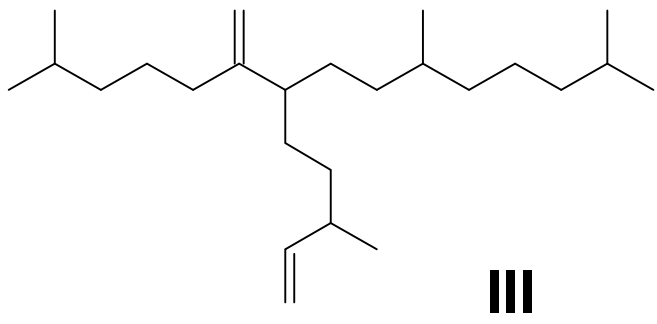
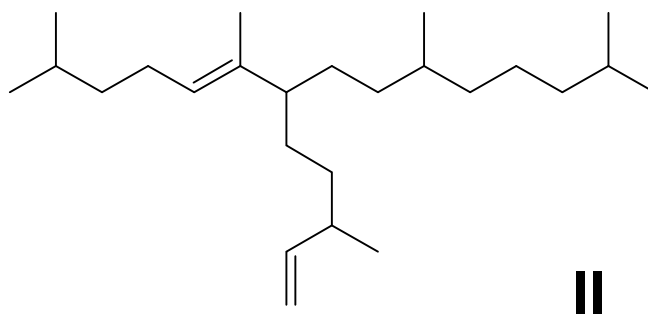
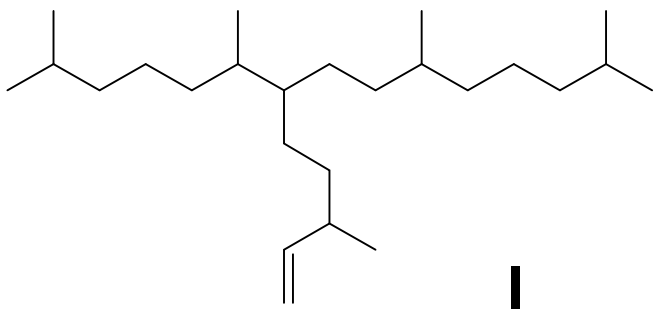
533 Figure 3. Mean H-Print ( $\pm$ SE) of algae samples containing sympagic and pelagic algae  
534 calculated using Eq. 2-5. Horizontal dashed lines show mean H-Prints calculated for samples  
535 containing 50% sympagic and 50% pelagic algae. Solid coloured lines are linear least squares  
536 regression fits to data points for each H-Print.

537

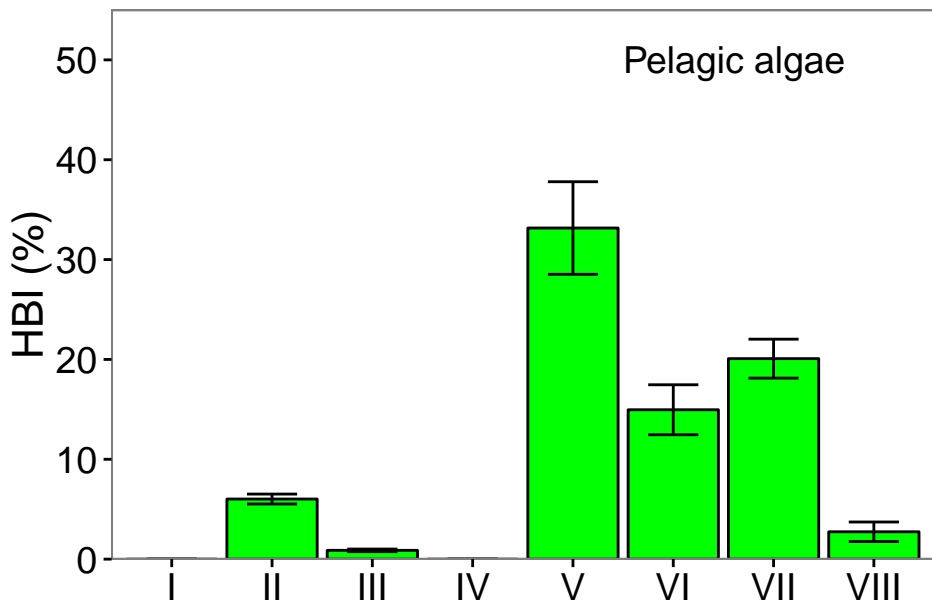
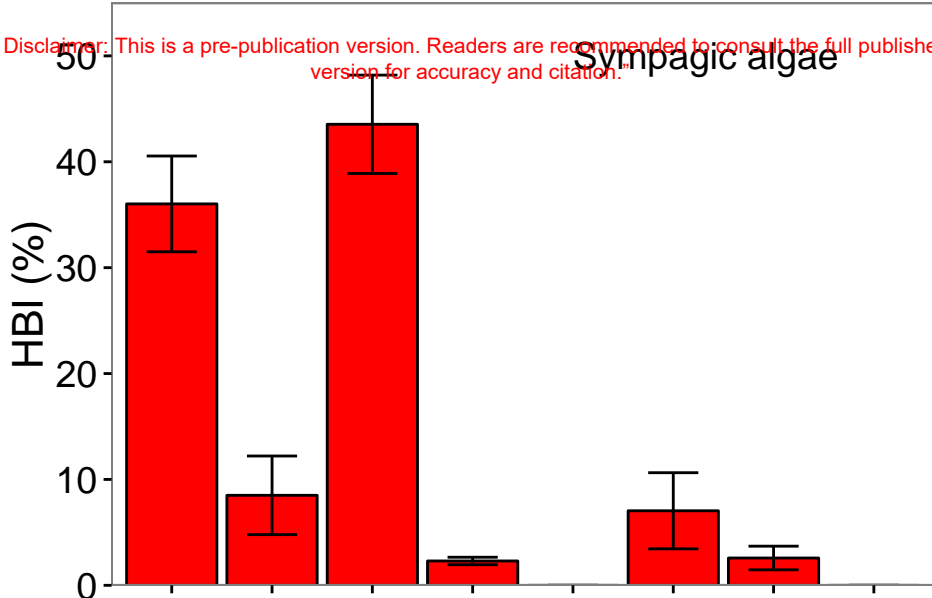
538 Figure 4. a) Mean H-Print<sup>3</sup> ( $\pm$ SE) of algae samples containing sympagic and pelagic algae.  
539 Straight line ( $R^2 = 0.97$ ;  $p = <0.001$ ) regression fit using H-Print<sup>3</sup> of food with 0.99  
540 confidence interval (SE) with regression formula shown. Horizontal and vertical dashed lines  
541 refer to 50:50 sympagic:pelagic and 50% H-Print. b) Mean H-Print<sup>3</sup> ( $\pm$ SE) of *Artemia* sp.  
542 (triangles) and filtered water (down triangles) calculated with H-Print<sup>3</sup>. Shaded area shows  
543 upper and lower CI from H-Print<sup>3</sup> algae regression model from panel a.

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