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

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Highlights

- A regression model defines the relationship between algal composition and H-Prints
- Algae H-Prints remain unchanged following grazing of algae by zooplankton
- H-Print offers quantitative estimates of sympagic /pelagic component of animal diet

Abstract

Quantifying the importance of sea ice microalgae as a food source in Arctic ecosystems is becoming an increasingly important research objective as sea ice extent and thickness continue to reduce. Recently, the analysis of certain diatom-derived highly branched isoprenoid (HBI) lipid biomarkers has provided a means of qualitatively distinguishing between sympagic (sea ice) and pelagic algae in Arctic animals. By combining the

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

50

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51 Keywords: Highly branched isoprenoid (HBI), H-Print, sea ice algae, sympagic carbon,

52 foodweb, Arctic

53

54

55

56 1 Introduction

57

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

68

69 Highly branched isoprenoid (HBI) lipids are secondary metabolites that have been reported in
70 a number of marine diatom genera that regularly form components of both sympagic and
71 pelagic algae. For instance, in the Arctic, the sea ice diatoms *Haslea crucigeroides*, *H.*
72 *spicula*, *H. kjellmanii* and *Pleurosigma stuxbergii* var *rhomboides* biosynthesise certain HBIs
73 (e.g. I and III; Fig. 1.; Brown et al., 2014c) that have a distinctive carbon isotopic
74 composition when found in the environment ($\delta^{13}\text{C} = -19\pm 2\text{‰}$ and $-18\pm 1\text{‰}$; Belt et al., 2008).
75 While these sympagic HBIs are regularly reported in samples of Arctic sea ice (Belt et al.,
76 2007, 2013; Brown et al., 2011), surface (Belt and Müller, 2013; Navarro-Rodriguez et al.,
77 2013; Smik et al., 2016; Stoyanova et al., 2013; Xiao et al., 2015) and downcore (Belt et al.,
78 2015; Müller et al., 2009, 2012; Polyak et al., 2016; Stein et al., 2016; Vare et al., 2009)
79 sediments, as yet, they have not been found in pelagic water samples from ice-free locations.
80 In contrast, other HBIs (including, II, IV, V, VI, VII and VIII; Fig. 1) are frequently reported

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

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

106

107 2 Material and methods

108

109 2.1 Diatom composition of sympagic and pelagic algae samples

110

111 For the determination of H-Print values in sympagic algae, samples of floating sea ice algae
112 aggregates were used that had been collected from Resolute Bay, Nunavut, Canada (Brown et
113 al., 2014c). Aggregates contained the HBI biosynthesising sea ice diatoms *H. crucigeroides*,
114 *H. spicula*, *H. kjellmanii*, *P. stuxbergii* var *rhomboides* (combined total ca. 2%), with the
115 remainder comprising mainly (ca. 80%) *Navicula pelagica*, *Pauliella taeniata* and *Nitzschia*
116 *frigida* (Brown et al., 2014c). Samples to represent pelagic algae were prepared by combining
117 cultures of known temperate HBI biosynthesising diatom species (*P. intermedium* (ca. 1%),
118 *P. planktonicum* (ca. 0.5%), *P. sp.* (ca. 1%) and *H. ostrearia* (ca. 2%)) with a non-HBI
119 producing centric diatom (*Thalassiosira weissfloggi*; ca. 95%). Total diatom cell abundances
120 in 10 mg subsamples were comparable for both sympagic (3.1×10^6 cell L⁻¹) and pelagic (3.5
121 $\times 10^6$ cell L⁻¹) algae.

122

123 2.2 Rearing and feeding of *Artemia* sp. - experimental setup

124

125 For feeding experiments, *Artemia* sp. were chosen since the *Artemiidae* are considered to be
126 continuous, non-selective filter feeders (Evjemo and Olsen, 1999), reaching saturated
127 ingestion capacity between 2×10^3 and 1×10^4 cells L⁻¹ (da Costa et al., 2005; Reeve, 1963),
128 which is below the cell abundances used here. About 1000 decapsulated *Artemia* sp. eggs
129 (Waterlife Research ind. Ltd.) were rinsed with deionised water (ca. 10 mL), transferred to
130 1.5 L clear plastic flasks (Corning) containing artificial seawater with a salinity of 32 (1 L
131 milli-Q water; 32 g TropicMarin® salt), and maintained in suspension by aeration. During

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132 rearing, light intensity was maintained below $1 \mu\text{mol photons m}^{-2} \text{s}^{-1}$. From 3 days after
133 hatching, a regime of 100% water changes, immediately followed by feeding (ca. 0.5 g
134 $\text{H}^2\text{Ocean}^{\text{Pro+}}$), was carried out every 2–3 days. At >40 days old, 20 individual *Artemia* sp.
135 were selected randomly from the rearing stock and transferred, along with 10 mg algae, to
136 experimental flasks containing artificial seawater. Experimental flasks were maintained at
137 $20 \pm 2.5^\circ\text{C}$ and ca. $2 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, with algae sustained in suspension by vigorous
138 aeration. After feeding on algae for 24 h, *Artemia* sp. were removed from the water using a
139 plastic pipette and pooled ($n=20$) for analysis of HBI content. The remaining water
140 containing ungrazed algae and faecal pellets was filtered (Whatman GF/F) and analysed for
141 HBI content.

142

143 2.3 Extraction and analysis of HBI lipids

144

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

149

150 2.4 Calculation of the HBI biomarker H-Print

151

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

157 both sympagic and pelagic diatoms to provide H-Print values within the range 0–100% (Eq.
158 1).

159

160

Eq. 1.

$$161 \quad H - \text{Print} (\%) = \frac{(\text{pelagic HBIs})}{(\text{sympagic HBIs} + \text{pelagic HBIs})} \times 100$$

162

163 To represent pelagic algae, 4 combinations of HBIs were tested. Firstly, for H-Print¹, all HBI
164 isomers, for which the chemical structures and at least one biological source organism were
165 also known (Eq. 2), were used. For H-Print² (Eq. 3), only V and VI were used, since these are
166 the most common HBIs in phytoplankton (Belt et al., 2000). H-Print³⁻⁴ used only V, as it was
167 absent from the sympagic algae (Eq. 4). To represent biomarkers of sympagic algae, I and III
168 were chosen for H-Prints¹⁻³ (Eq. 2–4) since these are the only HBIs in this study that are
169 known to have a sea ice diatom source (Brown et al., 2014c). For H-Print⁴ (Eq. 5) only I was
170 included as a sympagic component since it was not present in the pelagic algae.

171

Eq. 2.

$$172 \quad H - \text{Print}^1 (\%) = \frac{(\text{II} + \text{IV} + \text{V} + \text{VI} + \text{VII} + \text{VIII})}{(\text{I} + \text{II} + \text{III} + \text{IV} + \text{V} + \text{VI} + \text{VII} + \text{VIII})} \times 100$$

173

Eq. 3.

$$174 \quad H - \text{Print}^2 (\%) = \frac{(\text{V} + \text{VI})}{(\text{I} + \text{III} + \text{V} + \text{VI})} \times 100$$

175

Eq. 4.

$$176 \quad H - \text{Print}^3 (\%) = \frac{(\text{V})}{(\text{I} + \text{III} + \text{V})} \times 100$$

177

Eq. 5

$$178 \quad H - \text{Print}^4 (\%) = \frac{(\text{V})}{(\text{I} + \text{V})} \times 100$$

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179

180 2.5 Statistical design

181

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

191

192 4 Results

193

194 4.1 Quantification of HBIs and H-Prints in algae

195

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

210

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

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

220

221 4.2 H-Prints from analysis of *Artemia* sp. and filtered water samples

222

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

228

229 5. Discussion

230

231 5.1 Selection of HBIs for use in the H-Print

232

233 In order to make comparisons of food source (as defined by the H-Print) in grazers more
234 achievable, it was necessary to first identify the model that gave the most accurate
235 relationship between H-Prints and the known algal composition. This selection focused on
236 two main criteria; 1) accuracy of estimates and 2) confidence of the model. The most accurate
237 model was derived from H-Print³ using I, III and V as the constituent HBIs, which estimated
238 the sympagic and pelagic end-members at 101% and -1%, respectively (Table 1). In contrast,
239 models derived from the other H-Prints (1, 2 and 4) overestimated the sympagic component
240 at >109% and >4% for the sympagic and pelagic end-members, respectively. In assessing the
241 confidence intervals of the models, it was also found that the model using H-Print³ had the
242 smallest mean confidence interval range (29; Table 1), indicating the least uncertainty of all
243 the models, while the remaining models had mean confidence interval ranges >37. The linear
244 model derived from H-Print³ was therefore used to predict sympagic/pelagic algae
245 composition from hereon.

246

247 5.2 Comparison of H-Prints in algae, filtered water and *Artemia* sp.

248

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

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254 the H-Print. During the feeding experiments, there were no mortalities of *Artemia* sp., and the
255 consistent, rapid appearance of visible faecal pellets indicated that non-selective grazing
256 occurred for all experimental treatments. Experiments were run over 24 h to give *Artemia* sp.
257 suitable opportunity to consume algae, and it is estimated that *Artemia* sp. consumed ca.
258 150% of the available algae during each experiment. This was determined by comparing the
259 amount of HBI I in the 10 mg sympagic algae supplied to *Artemia* sp. (ca. 0.59 µg), with that
260 in pooled *Artemia* sp. after 24 h (ca. 0.02 µg), which indicated that ca. 3% of the algae was
261 present in the guts of *Artemia* sp. Based on an assumption of a gut passage time of 20–30
262 minutes (Nimura, 1989), it is estimated that ca. 50 gut passages per individual (ca. 1000 for
263 20 individuals) occurred over the duration of each experiment, potentially resulting in 15 mg
264 (150%) algae being consumed. This outcome is supported by the experiments of Reeve
265 (1963) who showed that *Artemia* sp. consumed *Phaeodactylum tricornutum* at ca. 4×10^5 cells
266 h^{-1} , which, over 24 h, corresponds to ca. 300% of our $3.1 \times 10^6 \text{ cell L}^{-1}$, and seems quite
267 feasible given the tendency for coprophagy in captive zooplankton (Werner, 2000). Despite
268 the efficient grazing of sympagic algae in the current experiments, *Artemia* sp. did not alter
269 the relative distributions of individual HBI biomarkers. Indeed, the majority (80%) of mean
270 H-Prints derived from water and *Artemia* sp. fell within the 99% confidence interval of the
271 regression model calculated using the algal H-Print³ (Fig. 4b), suggesting that *Artemia* sp.
272 grazed non-selectively on all treatments, without alteration of the H-Print. It is concluded,
273 therefore, that the regression model, based upon algal H-Prints, remains accurate for *Artemia*
274 sp., permitting application of the regression model to predict the sympagic/pelagic proportion
275 of zooplankton diet.

276

277 5.3 Environmental application of the H-Print

278

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279 Although it has been shown here that the H-Print approach can yield reliable estimates of the
280 sympagic/pelagic composition of algae in the laboratory, even after being consumed by
281 zooplankton, it is probably also important to consider some additional factors that may be
282 important when using this method in environmental settings in the future. Three such factors
283 are considered briefly here. Firstly, HBIs should be readily detectable in the environment.
284 Recently, the ubiquity of HBIs has been established following the widespread reporting of
285 these lipids in, for example, sea ice (Belt et al., 2007,2013,2016; Brown et al., 2011; Massé et
286 al., 2011; Nichols et al., 1988), sediments (Belt and Müller, 2013; Navarro-Rodriguez et al.,
287 2013; Xiao et al., 2015), zooplankton (Brown and Belt, 2012; Cripps, 1995), fish (Brown et
288 al., 2015; Goutte et al., 2014b), birds (Brown et al., 2013a,2015) and marine mammals
289 (Brown, et al., 2013a,2014a; Goutte et al., 2014a) from Arctic, Antarctic and temperate
290 environments. Second, it is suggested that identification of the H-Print formula that best
291 reflects the composition of natural sympagic and pelagic algae may also be important. In the
292 current study, it was shown that a key factor in identifying such a formula was selection of
293 HBIs that provide a balanced contribution from sympagic and pelagic sources. Achieving this
294 balance is, to some extent, simplified by the shared ability of sympagic and pelagic algae to
295 biosynthesise HBIs, thereby minimising any potential complications associated with
296 comparing lipids from different sources; a problem frequently associated with more common
297 lipids such as fatty acids or sterols which can have a wide range of sources (e.g. Natalia et al.,
298 1999; Volkman et al., 1986,1998), including, in some cases, animals themselves.
299 Nonetheless, the proportion of sympagic/pelagic HBIs will be dependent upon the overall
300 abundances of HBI-biosynthesising diatoms present. On this basis, it is anticipated that H-
301 Print³ may, as it did in the experiments described herein, provide the most realistic estimates
302 for environmental samples, since the species known to produce I, III and V have similar
303 abundances in the environment. For example, the diatoms that produce I and III have a

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304 consistent abundance in sea ice throughout the Arctic (1–5%; Brown et al., 2014c and
305 references therein), while *Pleurosigma* spp. and *Rhizosolenia* spp. (known sources of V) are
306 also typically 1–5% of species in pelagic diatom assemblages (Mather et al., 2010; von
307 Quillfeldt, 2000). On the other hand, if the abundances of the sympagic or pelagic sources
308 should differ substantially from this, it is possible that H-Print³ may not be the best predictor
309 of algal composition and a different combination of HBIs may give more reliable estimates.
310 In order to obtain the most reliable estimates of algal composition in samples collected from
311 the environment, it is therefore recommended that H-Prints for both sympagic and pelagic
312 algae are determined on a case-by-case basis using samples collected from the environment
313 being studied. Accordingly, the appropriate model can thus be selected that best estimates the
314 composition of mixed algae samples.

315

316 Finally, differential modification of HBI lipid distributions within animals would likely result
317 in inaccurate estimates of algae composition, regardless of which H-Print formula is used. As
318 such, it is important that, once consumed by animals, HBIs do not become modified from
319 their original source distributions, as was the case for *Artemia* sp. described herein. In the
320 Arctic, the most abundant zooplankton are usually *Calanus* spp. (Auel and Hagen, 2002;
321 Søreide et al., 2008) and, in contrast to *Artemia* sp., these do accumulate lipids (Graeve et al.,
322 2005; Pond and Tarling, 2011). The close similarity of *Calanus* spp. H-Prints with those from
323 pelagic algae (Brown et al., 2014d), however, in addition to those between some higher
324 trophic level organisms and sympagic algae (Brown et al., 2014d), suggest that HBI
325 distributions are not noticeably impacted by animals. Indeed, quantitative assessment of the
326 relative proportions of I and III in over 300 ringed seals showed a consistent ratio (1:2.7) that
327 aligned closely to typical values from sea ice (e.g. 1:2.1 (Belt et al., 2013) and 1:2.9 (Brown,
328 2011)), further supporting the finding here that H-Prints are unlikely to be significantly

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

332

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

343

344

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349 References

- 350 Arrigo, K.R., 2014. Sea Ice Ecosystems. *Ann. Rev. Mar. Sci.* 6, 439–467.
- 351 Auel, H., Hagen, W., 2002. Mesozooplankton community structure, abundance and biomass
352 in the central Arctic Ocean. *Mar. Biol.* 140, 1013–1021.
- 353 Belt, S.T., Müller, J., 2013. The Arctic sea ice biomarker IP₂₅: a review of current
354 understanding, recommendations for future research and applications in palaeo sea ice
355 reconstructions. *Quat. Sci. Rev.* 79, 9–25.
- 356 Belt, S.T., Allard, W.G., Massé, G., Robert, J.M., Rowland, S.J., 2000. Highly branched
357 isoprenoids (HBIs): Identification of the most common and abundant sedimentary
358 isomers. *Geochim. Cosmochim. Acta* 64, 3839–3851.
- 359 Belt, S.T., Massé, G., Rowland, S.J., Poulin, M., Michel, C., LeBlanc, B., 2007. A novel
360 chemical fossil of palaeo sea ice: IP₂₅. *Org. Geochem.* 38, 16–27.
- 361 Belt, S.T., Massé, G., Vare, L.L., Rowland, S.J., Poulin, M., Sicre, M.-A., Sampei, M.,
362 Fortier, L., 2008. Distinctive ¹³C isotopic signature distinguishes a novel sea ice
363 biomarker in Arctic sediments and sediment traps. *Mar. Chem.* 112, 158–167.
- 364 Belt, S.T., Brown, T.A., Navarro-Rodriguez, A., Cabedo-Sanz, P., Tonkin, A., Ingle, R.,
365 2012. A reproducible method for the extraction, identification and quantification of
366 the Arctic sea ice proxy IP₂₅ from marine sediments. *Anal. Methods* 4, 705–713.
- 367 Belt, S.T., Brown, T.A., Ringrose, A.E., Cabedo-Sanz, P., Mundy, C.J., Gosselin, M., Poulin,
368 M., 2013. Quantitative measurement of the sea ice diatom biomarker IP₂₅ and sterols
369 in Arctic sea ice and underlying sediments: further considerations for palaeo sea ice
370 reconstruction. *Org. Geochem.* 62, 33–45.
- 371 Belt, S.T., Cabedo-Sanz, P., Smik, L., Navarro-Rodriguez, A., Berben, S.M.P., Knies, J.,
372 Husum, K., 2015. Identification of paleo Arctic winter sea ice limits and the marginal

- 373 ice zone: Optimised biomarker-based reconstructions of late Quaternary Arctic sea
374 ice. *Earth Planet. Sci. Lett.* 431, 127–139.
- 375 Belt, S.T., Smik, L., Brown, T.A., Kim, J.-H., Rowland, S.J., Allen, C.S., Gal, J.-K., Shin,
376 K.-H., Lee, J.I., Taylor, K.W.R., 2016. Source identification and distribution reveals
377 the potential of the geochemical Antarctic sea ice proxy IPSO₂₅. *Nat. Commun.*
378 7, 12655.
- 379 Brown, T.A., 2011. Production and preservation of the Arctic sea ice diatom biomarker IP₂₅.
380 PhD Thesis, School of Geography Earth and Environmental Sciences. University of
381 Plymouth, Plymouth.
- 382 Brown, T.A., Belt, S.T., Philippe, B., Mundy, C.J., Massé, G., Poulin, M., Gosselin, M.,
383 2011. Temporal and vertical variations of lipid biomarkers during a bottom ice diatom
384 bloom in the Canadian Beaufort Sea: Further evidence for the use of the IP₂₅
385 biomarker as a proxy for spring Arctic sea ice. *Polar Biol.* 34, 1857–1868.
- 386 Brown, T.A., Belt, S.T., 2012. Closely linked sea ice–pelagic coupling in the Amundsen Gulf
387 revealed by the sea ice diatom biomarker IP₂₅. *J. Plankton Res.* 34, 647–654.
- 388 Brown, T.A., Belt, S.T., Ferguson, S.H., Yurkowski, D.J., Davison, N.J., Barnett, J.E.F.,
389 Jepson, P.D., 2013a. Identification of the sea ice diatom biomarker IP₂₅ and related
390 lipids in marine mammals: A potential method for investigating regional variations in
391 dietary sources within higher trophic level marine systems. *J. Exp. Mar. Biol. Ecol.*
392 441, 99–104.
- 393 Brown, T.A., Bicknell, A.W.J., Votier, S.C., Belt, S.T., 2013b. Novel molecular
394 fingerprinting of marine avian diet provides a tool for gaining insights into feeding
395 ecology. *Environ. Chem. Lett.* 11, 283–288.
- 396 Brown, T.A., Alexander, C., Yurkowski, D.J., Ferguson, S., Belt, S.T., 2014a. Identifying
397 variable sea ice carbon contributions to the Arctic ecosystem: A case study using

- 398 highly branched isoprenoid lipid biomarkers in Cumberland Sound ringed seals.
399 *Limnol. Oceanogr.* 59, 1581–1589.
- 400 Brown, T.A., Belt, S.T., Cabedo-Sanz, P., 2014b. Identification of a novel di-unsaturated C₂₅
401 highly branched isoprenoid in the marine tube-dwelling diatom *Berkeleya rutilans*.
402 *Environ. Chem. Lett.* 12, 455–460.
- 403 Brown, T.A., Belt, S.T., Tatarek, A., Mundy, C.J., 2014c. Source identification of the Arctic
404 sea ice proxy IP₂₅. *Nat. Commun.* 5, 4197.
- 405 Brown, T.A., Yurkowski, D.J., Ferguson, S.H., Alexander, C., Belt, S.T., 2014d. H-Print: a
406 new chemical fingerprinting approach for distinguishing primary production sources
407 in Arctic ecosystems. *Environ. Chem. Lett.* 12, 387–392.
- 408 Brown, T.A., Hegseth, E.N., Belt, S.T., 2015. A biomarker-based investigation of the mid-
409 winter ecosystem in Rijpfjorden, Svalbard. *Polar Biol.* 38, 37–50.
- 410 Brown, T.A., Belt, S.T., 2016. Novel tri- and tetra-unsaturated highly branched isoprenoid
411 (HBI) alkenes from the marine diatom *Pleurosigma intermedium*. *Org. Geochem.* 91,
412 120–122.
- 413 Cooke, D.A., Barlow, R., Green, J., Belt, S.T., Rowland, S.J., 1998. Seasonal variations of
414 highly branched isoprenoid hydrocarbons and pigment biomarkers in intertidal
415 sediments of the Tamar Estuary, UK. *Mar. Environ. Res.* 45, 309–324.
- 416 Cripps, G.C., 1995. The occurrence of monounsaturated n-C₂₁ and polyunsaturated C₂₅
417 sedimentary hydrocarbons in the lipids of Antarctic marine organisms. *Polar Biol.* 15,
418 253–259.
- 419 da Costa, R.A.A.M., Koenig, M.L., Pereira, L.C.C., 2005. Feeding adult of *Artemia salina*
420 (Crustacea-Branchiopoda) on the dinoflagellate *Gyrodinium corsicum*
421 (Gymnodiniales) and the Chryptophyta *Rhodomonas baltica*. *Braz. Arch. Biol.*
422 *Technol.* 48, 581–587.

- 423 Dieckmann, G.S., Hellmer, H.H., 2010. The importance of sea ice: An overview. In: Thomas,
424 D., Dieckmann, S. (Eds.), Sea ice (second edition). Blackwell Publishing, Chichester,
425 pp. 1–22.
- 426 Dunlop, R.W., Jefferies, P.R., 1985. Hydrocarbons of the hypersaline basins of Shark Bay,
427 Western-Australia. *Org. Geochem.* 8, 313–320.
- 428 Evjemo, J.O., Olsen, Y., 1999. Effect of food concentration on the growth and production
429 rate of *Artemia franciscana* feeding on algae (T. iso). *J. Exp. Mar. Biol. Ecol.* 242,
430 273–296.
- 431 Goutte, A., Charrassin, J.B., Cherel, Y., Carravieri, A., De Grissac, S., Masse, G., 2014a.
432 Importance of ice algal production for top predators: new insights using sea-ice
433 biomarkers. *Mar. Ecol. Prog. Ser.* 513, 269–275.
- 434 Goutte, A., Cherel, Y., Ozouf-Costaz, C., Robineau, C., Lanshere, J., Massé, G., 2014b.
435 Contribution of sea ice organic matter in the diet of Antarctic fishes: a diatom-specific
436 highly branched isoprenoid approach. *Polar Biol.* 37, 903–910.
- 437 Graeve, M., Albers, C., Kattner, G., 2005. Assimilation and biosynthesis of lipids in Arctic
438 *Calanus* species based on feeding experiments with a ¹³C labelled diatom. *J. Exp.*
439 *Mar. Biol. Ecol.* 317, 109–125.
- 440 Grossi, V., Beker, B., Geenevasen, J.A.J., Schouten, S., Raphel, D., Fontaine, M.-F.,
441 Sinninghe Damsté, J.S., 2004. C₂₅ highly branched isoprenoid alkene from the marine
442 benthic diatom *Pleurosigma strigosum*. *Phytochemistry* 65, 3049–3055.
- 443 He, D., Simoneit, B.R.T., Xu, Y., Jaffé, R., 2016. Occurrence of unsaturated C₂₅ highly
444 branched isoprenoids (HBIs) in a freshwater wetland. *Org. Geochem.* 93, 59–67.
- 445 Kaiser, J., Belt, S.T., Tomczak, M., Brown, T.A., Wasmund, N., Arz, H.W., 2016. C₂₅ highly
446 branched isoprenoid alkenes in the Baltic Sea produced by the marine planktonic
447 diatom *Pseudosolenia calcar-avis*. *Org. Geochem.* 93, 51–58.

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- 448 Massé, G., Belt, S.T., Crosta, X., Schmidt, S., Snape, I., Thomas, D.N., Rowland, S.J., 2011.
449 Highly branched isoprenoids as proxies for variable sea ice conditions in the Southern
450 Ocean. *Antarct. Sci.* 23, 487–498.
- 451 Mather, L., MacIntosh, K., Kaczmarska, I., Klein, G., Martin, J.L., 2010. A checklist of
452 diatom species reported (and presumed native) from Canadian coastal waters. *Can.*
453 *Tech. Rep. Fish. Aquat. Sci.* 1–78.
- 454 Meier, W.N., Hovelsrud, G.K., van Oort, B.E.H., Key, J.R., Kovacs, K.M., Michel, C., Haas,
455 C., Granskog, M.A., Gerland, S., Perovich, D.K., Makshtas, A., Reist, J.D., 2014.
456 Arctic sea ice in transformation: A review of recent observed changes and impacts on
457 biology and human activity. *Rev. Geophys.* 2013RG000431.
- 458 Müller, J., Massé, G., Stein, R., Belt, S.T., 2009. Variability of sea-ice conditions in the Fram
459 Strait over the past 30,000 years. *Nat. Geosci.* 2, 772–776.
- 460 Müller, J., Werner, K., Stein, R., Fahl, K., Moros, M., Jansen, E., 2012. Holocene cooling
461 culminates in sea ice oscillations in Fram Strait. *Quat. Sci. Rev.* 47, 1–14.
- 462 Natalia, V. Z., and I. K. Vladimir. 1999. Sources of essential fatty acids in the marine
463 microbial loop. *Aquat. Microb. Ecol.* 17, 153–157.
- 464 Navarro-Rodriguez, A., Belt, S.T., Knies, J., Brown, T.A., 2013. Mapping recent sea ice
465 conditions in the Barents Sea using the proxy biomarker IP₂₅: implications for palaeo
466 sea ice reconstructions. *Quat. Sci. Rev.* 79, 26–39.
- 467 Nichols, P.D., Palmisano, A.C., Volkman, J.K., Smith, G.A., White, D.C., 1988. Occurrence
468 of an isoprenoid C₂₅ diunsaturated alkene and high neutral lipid content in Antarctic
469 sea-ice diatom communities. *J. Phycol.* 24, 90–96.
- 470 Nimura, Y., 1989. Shortest gut passage time and gut content volume of *Artemia franciscana*.
471 *Nippon Suisan Gakkaishi* 55, 2209–2209.

Disclaimer: This is a pre-publication version. Readers are recommended to consult the full published version for accuracy and citation.

- 472 Polyak, L., Belt, S.T., Cabedo-Sanz, P., Yamamoto, M., Park, Y.-H., 2016. Holocene sea-ice
473 conditions and circulation at the Chukchi-Alaskan margin, Arctic Ocean, inferred
474 from biomarker proxies. *Holocene* 26, 1810–1821.
- 475 Pond, D.W., Tarling, G.A., 2011. Phase transitions of wax esters adjust buoyancy in
476 diapausing *Calanoides acutus*. *Limnol. Oceanogr.* 56, 1310–1318.
- 477 R-Core-Team, 2016. R: A language and environment for statistical computing, R Foundation
478 for Statistical Computing, Vienna, Austria, pp. URL <http://www.R-project.org/>.
- 479 Reeve, M.R., 1963. The Filter-Feeding of *Artemia*. I. In *Pure Cultures of Plant Cells* 40, 195–
480 205.
- 481 Rowland, S.J., Allard, W.G., Belt, S.T., Massé, G., Robert, J.M., Blackburn, S., Frampton,
482 D., Reville, A.T., Volkman, J.K., 2001. Factors influencing the distribution of
483 polyunsaturated terpenoids in the diatom, *Rhizosolenia setigera*. *Phytochemistry* 58,
484 717–728.
- 485 Smik, L., Cabedo-Sanz, P., Belt, S.T., 2016. Semi-quantitative estimates of paleo Arctic sea
486 ice concentration based on source-specific highly branched isoprenoid alkenes: A
487 further development of the PIP₂₅ index. *Org. Geochem.* 92, 63–69.
- 488 Søreide, J.E., Falk-Petersen, S., Hegseth, E.N., Hop, H., Carroll, M.L., Hobson, K.A.,
489 Blachowiak-Samolyk, K., 2008. Seasonal feeding strategies of *Calanus* in the high-
490 Arctic Svalbard region. *Deep-Sea Res. Pt. II* 55, 2225–2244.
- 491 Stein, R., Fahl, K., Schreck, M., Knorr, G., Niessen, F., Forwick, M., Gebhardt, C., Jensen,
492 L., Kaminski, M., Kopf, A., Matthiessen, J., Jokat, W., Lohmann, G., 2016. Evidence
493 for ice-free summers in the late Miocene central Arctic Ocean. *Nat. Commun.* 7,
494 11148.
- 495 Stoyanova, V., Shanahan, T.M., Hughen, K.A., de Vernal, A., 2013. Insights into Circum-
496 Arctic sea ice variability from molecular geochemistry. *Quat. Sci. Rev.* 79, 63–73.

- 497 Stroeve, J.C., Kattsov, V., Barrett, A., Serreze, M., Pavlova, T., Holland, M., Meier, W.N.,
498 2012. Trends in Arctic sea ice extent from CMIP5, CMIP3 and observations.
499 Geophys. Res. Lett. 39.
- 500 Vare, L.L., Massé, G., Gregory, T.R., Smart, C.W., Belt, S.T., 2009. Sea ice variations in the
501 central Canadian Arctic Archipelago during the Holocene. Quat. Sci Rev. 28, 1354–
502 1366.
- 503 Volkman, J. K. 1986. A review of sterol markers for marine and terrigenous organic matter.
504 Org. Geochem. 9, 83–99.
- 505 Volkman, J. K., Barrett, S.M., Blackburn, S.I., Mansour, M.P., Sikes, E.L., Gelin, F., 1998.
506 Microalgal biomarkers: A review of recent research developments. Org. Geochem.
507 29, 1163–1179.
- 508 Volkman, J.K., Barrett, S.M., Dunstan, G.A., 1994. C₂₅ and C₃₀ highly branched isoprenoid
509 alkenes in laboratory cultures of two marine diatoms. Organic Geochemistry 21, 407-
510 414.
- 511 von Quillfeldt, C.H., 2000. Common Diatom Species in Arctic Spring Blooms: Their
512 Distribution and Abundance, Botanica Marina, pp. 499.
- 513 Werner, I., 2000. Faecal pellet production by Arctic under-ice amphipods–transfer of organic
514 matter through the ice/water interface. Hydrobiologia 426, 89–96.
- 515 Wraige, E.J., Belt, S.T., Lewis, C.A., Cooke, D.A., Robert, J.M., Massé, G., Rowland, S.J.,
516 1997. Variations in structures and distributions of C₂₅ highly branched isoprenoid
517 (HBI) alkenes in cultures of the diatom, *Haslea ostrearia* (Simonsen). Org. Geochem.
518 27, 497–505.
- 519 Xiao, X., Fahl, K., Müller, J., Stein, R., 2015. Sea-ice distribution in the modern Arctic
520 Ocean: Biomarker records from trans-Arctic Ocean surface sediments. Geochim.
521 Cosmochim. Acta 155, 16–29.

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522 Xu, Y., Jaffé, R., Wachnicka, A., Gaiser, E.E., 2006. Occurrence of C₂₅ highly branched
523 isoprenoids (HBIs) in Florida Bay: Paleoenvironmental indicators of diatom-derived
524 organic matter inputs. *Org. Geochem.* 37, 847–859.

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

	<i>H-Print</i> <i>Eq.#</i>	<i>R</i> ²	<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	
Composition (S:P)									
H-Print¹	2	0.95	8.6	23	<0.01	4 (-14 – 23)	59 (41 – 77)	113 (95 – 133)	37
H-Print²	3	0.92	10.4	23	<0.01	6 (-16 – 29)	67 (45 – 89)	129 (104 – 153)	46
H-Print³	4	0.97	6.6	23	<0.01	-1 (-16 – 14)	50 (36 – 64)	101 (87 – 116)	29
H-Print⁴	5	0.89	11.7	23	<0.01	13 (-11 – 39)	61 (37 – 86)	109 (83 – 135)	50

529

530

531 Figure legends

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

534

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

537

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

542

543 Figure 4. a) Mean H-Print³ (\pm SE) of algae samples containing sympagic and pelagic algae.

544 Straight line ($R^2 = 0.97$; $p = <0.001$) regression fit using H-Print³ of food with 0.99

545 confidence interval (SE) with regression formula shown. Horizontal and vertical dashed lines

546 refer to 50:50 sympagic:pelagic and 50% H-Print. b) Mean H-Print³ (\pm SE) of *Artemia* sp.

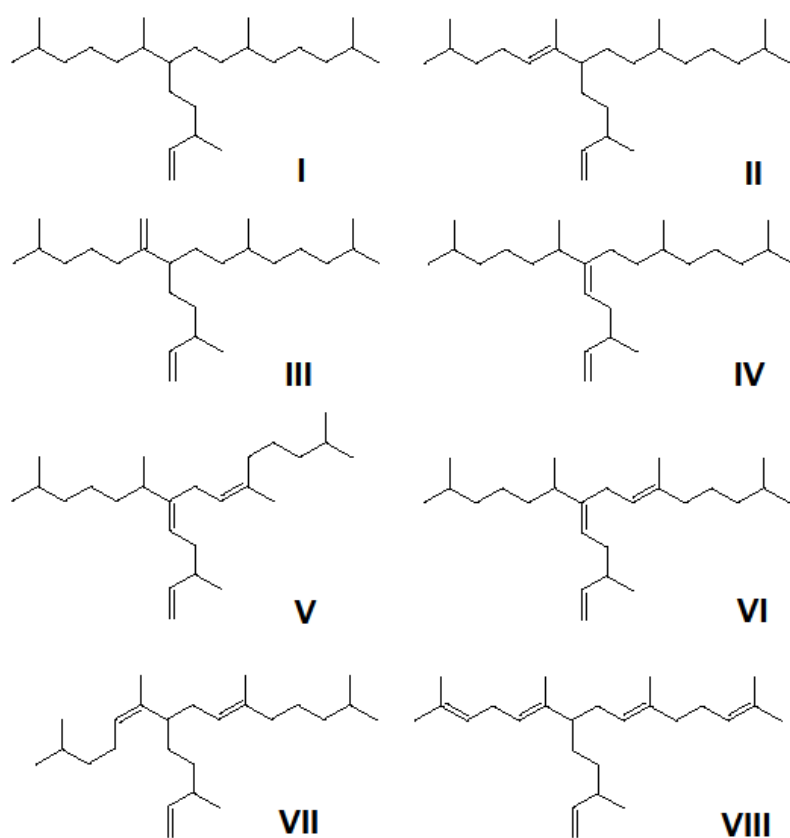
547 (triangles) and filtered water (down triangles) calculated with H-Print³. Shaded area shows

548 upper and lower CI from H-Print³ algae regression model from panel a.

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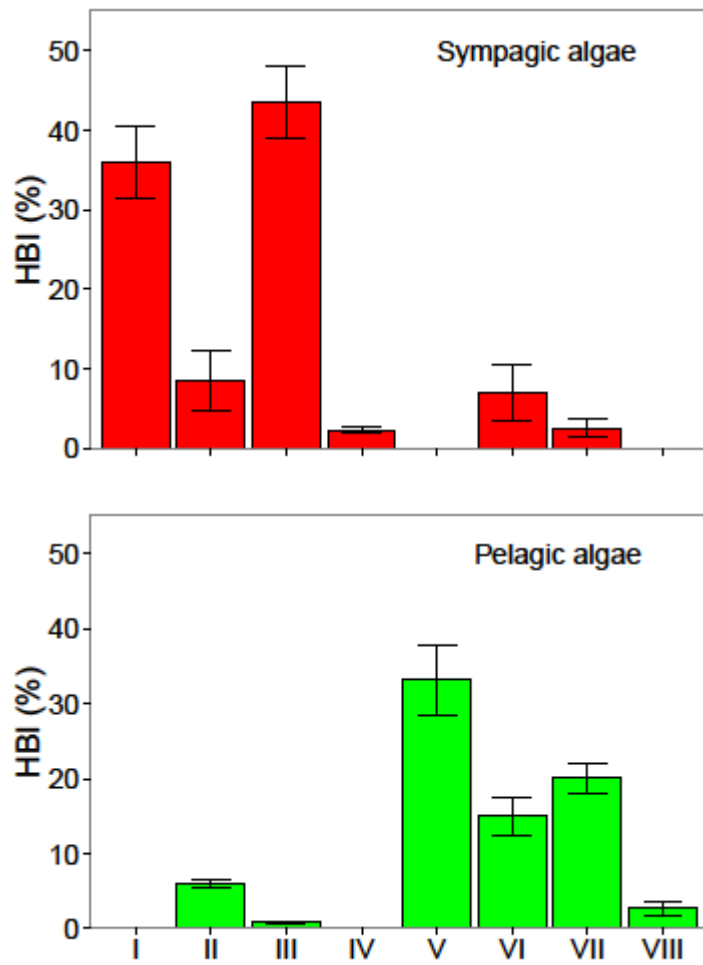
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552 Fig. 1

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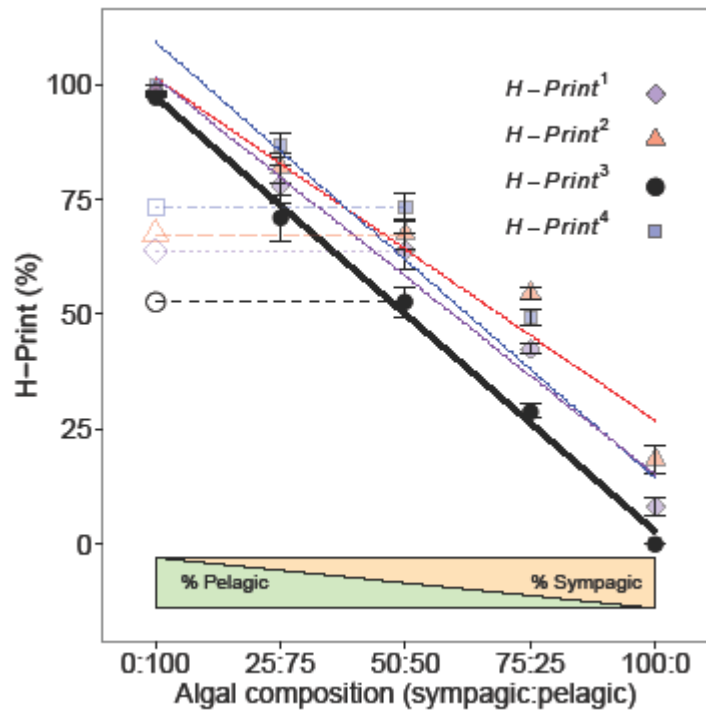
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554 Fig. 2

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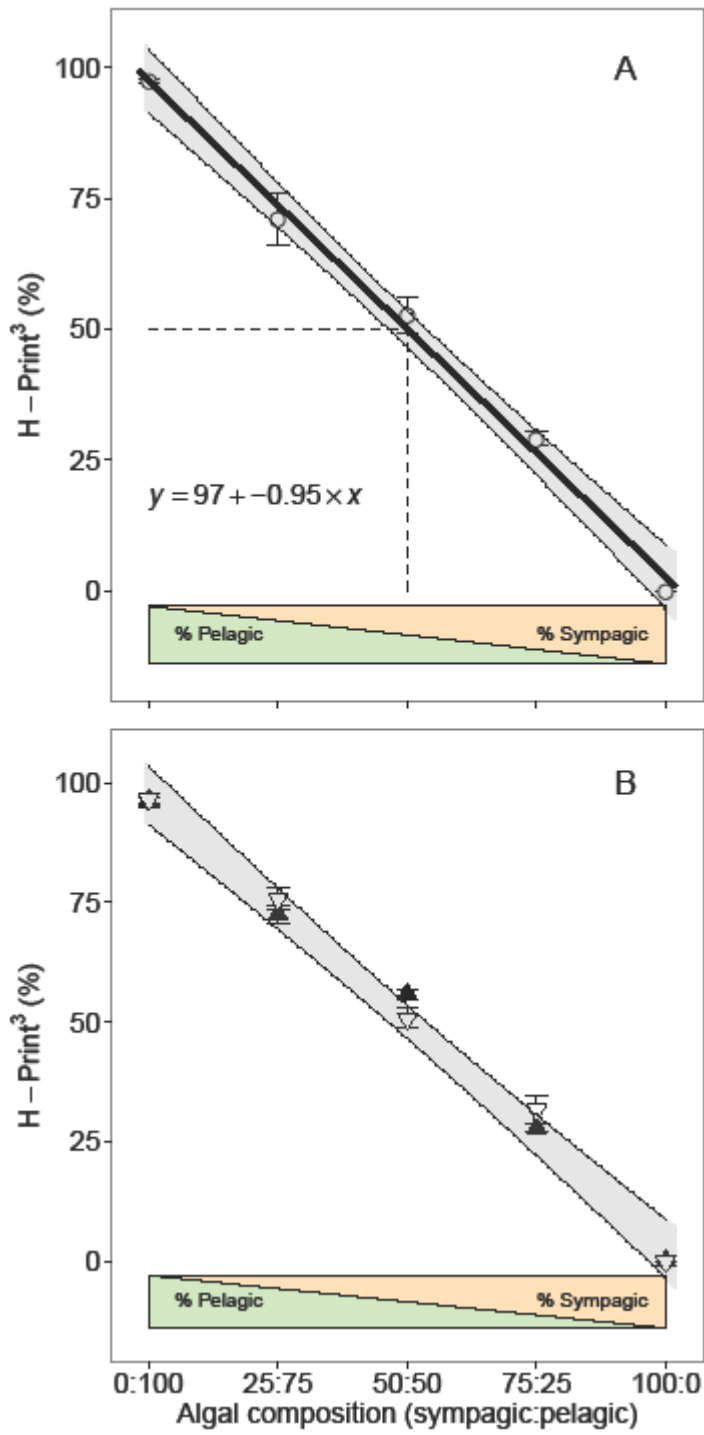


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558 Fig. 3

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561 Fig. 4

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