Chloroplast DNA methylation in the kelp *Saccharina latissima* is determined by origin and influenced by cultivation

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Abstract

DNA cytosine methylation is an important epigenetic mechanism in genomic DNA, but absent in the chloroplast DNA of most land plants. We detected methylation in the chloroplast DNA of the kelp *Saccharina latissima*, a non-model macroalgal species of high ecological (wild populations) and economic importance. Since the functional role of the chloroplast methylome is yet largely unknown, we compared for the first time the chloroplast DNA cytosine methylation between wild and cultured kelp from different climatic origins (High-Arctic (79 °N) and temperate (54 °N), laboratory samples at 5 °C, 10 °C and 15 °C). Our results suggest genome-wide differences in methylated sites, and methylation level, between the origins, and a strong effect of cultivation. Functions related to photosynthesis showed differential methylation only between origins. Significant differences in methylation between cultivated and wild samples of genes related to transcription and translation were unique to

the high-arctic samples. Both findings indicate that origin and cultivation strongly, but differently affect the chloroplast methylome. Similar methylomes for samples from the same origin – independent from whether they grow in the wild or in the lab – suggest that origin-specific methylation marks on the chloroplast genome are inherited. Given that DNA-methylation affects gene expression, our study suggests that lab-cultivation alters epigenetically determined kelp chloroplast characteristics at least to the same degree as ecotypic differentiation does, but likely on a much shorter timescale. This indicates the capacity for rapid non-genetic adaptation in the kelp *Saccharina latissima*.

Keywords:

Chloroplast, organelle genome methylation, epigenetics, non-model organism, cultivation, aquaculture, marine macrophyte, marine algae

Introduction

Primary production, by which photoautotrophic organisms convert oxidised inorganic carbon into organic carbon via photosynthesis, is the one crucial process that ensures the existence of nearly all food webs, both terrestrial and marine. Since chloroplasts are presumed to have developed from a free-living cyano-bacteria to an endosymbiontic organelle (Nishimura 2010), they feature their own, albeit reduced, genome. Due to the reduced nature of their genome, the organelles are semi-autonomous, and depend on extra-organellar products for their internal processes (Wang et al. 2020). Epigenetic mechanisms include histone modification, non-coding RNA, and DNA methylation (Boquete et al. 2021). They have been shown to play vital roles in many cellular processes, such as regulation of gene expression, mRNA processing, timing of DNA replication, determination of chromatin structure, or silencing of transposons (Finnegan et al. 1998; Zhang et al. 2018). DNA cytosine methylation is the most stable epigenetic mechanism that can persist for multiple generations (Lämke and Bäurle 2017), thus

52 is presumed to be most relevant for epigenetic adaptation. In contrast, histone modifications
53 and ncRNAs are often only stable for several days or weeks (Lämke and Bäurle, 2017).
54 However, the function of the chloroplast genome methylation is yet unclear, but intensely
55 researched in the few species of mostly agricultural use that show chloroplast methylation,
56 such as tomato and rice, or the unicellular green alga Chlamydomonas (Kobayashi et al. 1990;
57 Nishimura et al. 1999; Muniandy et al. 2019). In marine primary producers, the first
58 chloroplast genome methylation has recently been documented in the brown macroalga
59 Saccharina japonica (Teng et al. 2021). Its functional relevance remains unknown.

60 Macroalgae are the predominant source of coastal marine primary production (Field et al.
61 1998; Filbee-Dexter 2020). Nearly exclusively restricted to the shorelines of the continents,
62 macroalgae act as foundation species of marine rocky shore ecosystems, in addition to
63 forming the base of food webs, and hence, have high and diverse ecological value (Bartsch et
64 al. 2008; Teagle et al. 2017; Duffy et al. 2019). Their economic value is increasing exponentially
65 as macroalgae cultivation gains momentum outside of Asia as an environmentally sustainable
66 new blue economy with a multitude of applications (Cai et al. 2021). In 2019, marine
67 macroalgae accounted for ~30% of the global production of marine aquaculture commodities
68 (120 million t), worth US$ 14.7 billion (Cai et al. 2021). However, in recent years macroalgae
69 distributions have shifted northward in response to increasing (summer) water temperatures
70 (Krause-Jensen et al. 2020; Bringloe et al. 2020). Heat waves affect both wild populations and
71 aquaculture yields. In kelp, the respective thermal tolerance of gameto- and sporophyte
72 stages seems to be the strongest factor defining their global distribution limits (Monteiro et
73 al. 2019; Liesner et al. 2020). Temperature is a controlling factor in many biological processes
74 such as photosynthetic carbon acquisition. Enzymatic activity of RubisCO, the carbon
75 capturing enzyme in photosynthesis, increases by a factor of 7 between 5°C and 15°C (Cen and

76 Sage 2005), and has been shown to be especially vulnerable to high temperatures. As the
77 chloroplast genome carries several photosynthesis-relevant enzymes, investigating its
78 methylation at different temperatures is particularly relevant when considering methylome-
79 based temperature adaptation in accordance to the eco-genotype approach (Scheschonk et
80 al. 2022). While epigenetic mechanisms have been extensively researched in terrestrial plants,
81 knowledge about kelp epigenetics still is limited to a handful of studies (Cock et al. 2010; Fan
82 et al. 2020a, b; Scheschonk et al. 2022), with a single study on chloroplast DNA methylation
83 (Teng et al. 2021). In primary producers, the chloroplast genome has been presumed to be
84 un-methylated, despite data suggesting otherwise (*Saccharina japonica*, Teng et al 2021;
85 *Chlamydomonas reinhardtii*, Nishimura 2010;). Epigenetic mechanisms hold the key to rapid
86 local adaptation, and hence the capacity for species survival and ecosystem stabilisation
87 despite swift alterations in biotic and abiotic factors. Especially in the face of the staggering
88 changes in local and global ecosystems due to the anthropogenic climate crisis, gaining
89 knowledge in the field of eco-evolutionary dynamics is crucial. Our study is the first attempt
90 to determine the influence of temperature, origin, and growth condition (wild *vs* cultivated)
91 on the methylome of chloroplasts of the kelp *Saccharina latissima* (sugar kelp). In the northern
92 hemisphere, this boreal-temperate marine brown alga, a congener species to *S. japonica* with
93 a large latitudinal distribution range, is among the macroalgal species of both high ecological
94 and economic value (Bartsch et al. 2008; Cai et al. 2021).

95 In this fundamental research study, we tested the following hypotheses:

96 1) *Saccharina latissima* features chloroplast genome methylations, as has been reported for
97 *S. japonica* (Teng et al 2021).

98 2) The chloroplast methylome mirrors the nuclear methylome (Scheschonk et al. 2022) in
99 showing epigenetic differentiation between origins that remains under cultivation, suggesting
100 a trans-generational epigenetic memory.

101 3) The chloroplast methylome changes in response to temperature and lab cultivation, thus
102 providing potential for rapid non-genetic adaptation to environmental change.

103 Materials and Methods

104 Laboratory cultures, field samples, and 'DNA- Extraction and MethlylRAD sequencing are
105 described in more detail in Scheschonk et al (2022). Changes and specific adjustments to the
106 present study were necessary during the bioinformatics and statistics part, and are detailed
107 below ('Bioinformatics and Statistics').

108 Laboratory cultures

109 As described in more detail in Scheschonk et al (2022), four cultures per origin (S1-S4, H1-H4;
110 technical replicates) were obtained from mono-parentally fertilised clonal gametophyte
111 cultures of *Saccharina latissima* (Linnaeus) C.E. Lane, C. Mayes, Druehl & G.W. Saunders from
112 Helgoland, German Bight (Nordstrand, 54°11'18.9"N 7°54'14.1"E; HG1, HG2, HG3, HG4 in
113 culture since 2014) and Spitsbergen, Svalbard (Hansneset, Kongsfjorden, 78°59'26.0"N
114 11°58'42.3"E; SG1, SG2, SG3, SG4 in culture since 2015; see table Suppl. Table 1) were kept at
115 each of the three temperatures (5 °C, 10 °C, and 15 °C) under white light at 15 µmol m-2 s-1
116 (18 : 6 hours light : dark) from as early as initiation of fertilization. Only sporophytes with a
117 fresh weight exceeding 120 mg (without stipe) were taken for DNA extraction.

118 Field Samples

119 Adult sporophytes were sampled from Helgoland, German Bight (54°11'18.9"N 7°54'14.1"E),
120 and from Spitsbergen, Svalbard (78°59'26.0"N 11°58'42.3"E) at the same locations from which
121 the gametophytes for the laboratory grown sporophytes originated. From each sporophyte, 4

122 discs (ø=3 cm) were cut from the fronds, omitting the meristematic region, 'midsection', and
123 reproductive tissues. Samples from lab and field were dried in cellulose bags stored in silica.
124 In total 21 samples from the laboratory cultures, and 20 samples from the wild were analysed.
125 In contrast to the field samples, our laboratory samples were uniparentally fertilised.
126 However, given that chloroplast genes are transmitted solely from the maternal parent
127 (matrilineally; Kuroiwa 1991, in Nishimura 2010), the chloroplast genome inheritance is
128 identical in uniparentally and bi-parentally fertilised individuals. This circumstance enables a
129 direct comparison between the chloroplast methylomes of the cultured and wild samples.

130 Bioinformatics and Statistics of MethylRAD sequencing
131

132 Clean-up of raw data

133 DNA was extracted and MethylRAD sequencing libraries were prepared as described in detail
134 in Scheschonk et al (2022). The sequences were de-multiplexed by sample and quality
135 trimmed with TrimGalore! v 0.4.1
136 (https://www.bioinformatics.babraham.ac.uk/projects/trim_galore/). Bases with Phred-
137 score <20 (--quality 20) were eliminated, the adapter sequence was removed (--stringency 3),
138 and the terminal 2bp (both ends) were removed to eliminate any artefacts at the ligation
139 position (--clip_R1 2 \ --three_prime_clip_R1 2). Reads were checked for fragment (over-)
140 representation, base AT CG content bias, and read length with FastQC v0.11.8 before and after
141 quality trimming.

142 In-silico digestion

143 For an overview of potential methylation sites, and as a backbone to map sequences to the
144 reference chloroplast genome for the *S. latissima* chloroplast (Fan et al. 2020b) was digested
145 *in silico* using the custom python script InSilicoTypeIIbDigestion_corrected.py
146 (http://marinetics.org/2017/04/11/REdigestions.html) and settings for simulating the

147 restriction enzyme FspEI. The *in silico* digestion found 270 recognition sites, where 69 were
148 CCGG recognition sites, 61 CCTGG sites, 77 CCTGT sites, and 63 CCAGG sites.

149 ## Mapping and annotation

150 The sequenced reads were mapped to the *in silico* digested reference genome of the
151 *S. latissima* chloroplast, combined with the *in silico* digested genome of the closely related
152 species *Saccharina japonica* to account for reads belonging to the nuclear genome and avoid
153 false mappings to the chloroplast genome for those reads. Reads were mapped using Burrows-
154 Wheeler Aligner (BWA Version: 0.7.17-r1188) (Li and Durbin 2010). Duplicate mappings were
155 excluded from further analyses by filtering out reads with a mapping quality <10 using
156 samtools (v 1.9). A count table was created listing the number of reads that mapped back to
157 each methylation site. For this, htseq-count (v 0.7.2) was called on each sample alignment
158 with *in silico* digested fragment in gff3 format as count features. An additional table was made
159 with counts normalized to 'reads per million (RPM) = (Reads mapped per site ×10^6)/(Total
160 number of mapped reads)' to account for varying sequencing depths.

161 Only methylated sites that were covered in at least one library with coverage >2 were kept in
162 count tables and RPM tables. The sites were annotated for their sequence context (CG or
163 CHG), genomic region, gene ID numbers, and gene functions based on the gff3 annotation file
164 for the *S. latissima* chloroplast genome from NCBI (Accession: MT151382.1) (Fan et al. 2020b).

165 ## Statistics

166 ### *Principal Component Analysis*

167 Differences in the chloroplast methylome were investigated with principal component
168 analysis (PCA) on RPM tables using the R package 'FactomineR' (Lê et al. 2008). Since lab and
169 field samples separated clearly into distinct groups in the PCA, their methylomes were
170 compared first for all samples, and then separated into origins (Helgoland and Spitsbergen).
171 Within the lab and field samples, and for all samples, we compared origins (Helgoland and

172 Spitsbergen). Within the lab samples, we compared between the treatment temperatures of
173 5°C, 10°C, and 15°C.

174 *Outlier detection*

175 The outlier function in the R package 'FactoInvestigate' (Thuleau & Husson, 2020) detected
176 two outliers, FH1 and FH9 (Helgoland field samples) in the PCA. The outliers showed few reads
177 mapping to the genome, few methylated sites, and high RPM values for some of the
178 methylated sites (Suppl. Fig. 1). Thus, FH1 and FH9 samples were excluded from all follow-up
179 analyses. No outliers were found in the Spitsbergen samples. As both outliers belonged to the
180 same group (Helgoland field samples), outlier removal led to a reduction in variance explained
181 by the first two principal components from 69.9% to 28.9% (Suppl. Figs. 1-3).

182 *Methylation levels*

183 We tested for differences in DNA methylation levels as RPM and for differences in the
184 numbers of methylated sites between populations, between treatment temperatures, and
185 between lab and field samples, using Wilcoxon rank sum tests. P-values were corrected with
186 the Benjamini-Hochberg method (Benjamini and Hochberg 1995) to control for the false
187 discovery rate, and results with adjusted p values padj <0.05 were considered significant. The
188 tests were performed using the R package 'rstatix' v0.7.0 (Kassambara, 2021).

189 We tested for correlation between the numbers of quality trimmed reads, and the numbers
190 of quality-trimmed reads that mapped back to the *Saccharina latissima* chloroplast genome
191 (Suppl. Fig. 2C, 3C, 2D, 3D).

192 *Differential methylation analysis*

193 We tested for significant differential methylation with the R package 'DESeq2' (Love et al.
194 2014) between 'Helgoland' *vs* 'Spitsbergen' in all samples ('All', or 'both locations'), and in only
195 field samples and only lab samples. Further, between 'field' *vs* 'lab' in all samples, and in only
196 Helgoland samples, and only Spitsbergen samples. We also compared pairwise between

197 temperature treatments: both origins combined (Helgoland + Spitsbergen 5 °C *vs* 10 °C; 5 °C
198 *vs* 15 °C; 10 °C *vs* 15 °C), separately for Helgoland and Spitsbergen (5 °C *vs* 10 °C; 5 °C *vs* 15 °C;
199 10 °C *vs* 15 °C), and for each temperature (Helgoland vs Spitsbergen @ 5 °C; 10 °C; 15 °C).
200 'DESeq2' normalised raw methylation counts before testing for differential methylation, and
201 corrected p-values with the Benjamini-Hochberg method for controlling false discovery rates
202 (Benjamini & Hochberg, 1995). Methylated sites with adjusted p-value padj < 0.05 were
203 considered significantly differentially methylated.

204 *GO term analysis*

205 Gene ontology (GO) terms for biological processes, cellular components, and molecular
206 functions were extracted from the database https://www.uniprot.org/ using Gene ID numbers
207 (Suppl. Table 2-5). The R package 'topGO' (Alexa & Rahnenführer, 2009) was used to test for
208 enrichment of the GO terms.

209 *Gene names and functions*

210 Names and functions of differentially methylated genes were extracted from the chloroplast
211 genome of *Saccharina latissima* published on NCBI (strain y-c14;
212 https://www.ncbi.nlm.nih.gov/nuccore/MT151382.1?report=graph).

213 *Analysis of epigenetic distance*

214 Epigenetic distances among lab and field samples (cultivation) or among samples from
215 Helgoland vs Spitsbeergen (origin) were estimated as euclidean distances among samples in
216 the PCA . T. The Shapiro-Wilk indicated non-normal distribution for groups analysed for
217 cultivation (p-value lab $p < 0.0001$, field $p < 0.0001$) and for origin (Helgoland $p < 0.0001$,
218 Spitsbergen $p < 0.0001$). Hence, we tested for differences in epigenetic distances among the
219 cultivation or origin categories using non-parametric Wilcox-rank-sum test (suppl. Table 6).

220 # Results

221 ## *Methylation levels and methylated sites*

222 *In-silico* digestion identified 69 MethylRAD sites for CG methylation, and 201 for CHG
223 methylation in the chloroplast genome of *Saccharina latissima*. 20.7% and 24.8% of these sites
224 were methylated, respectively (Table 1).

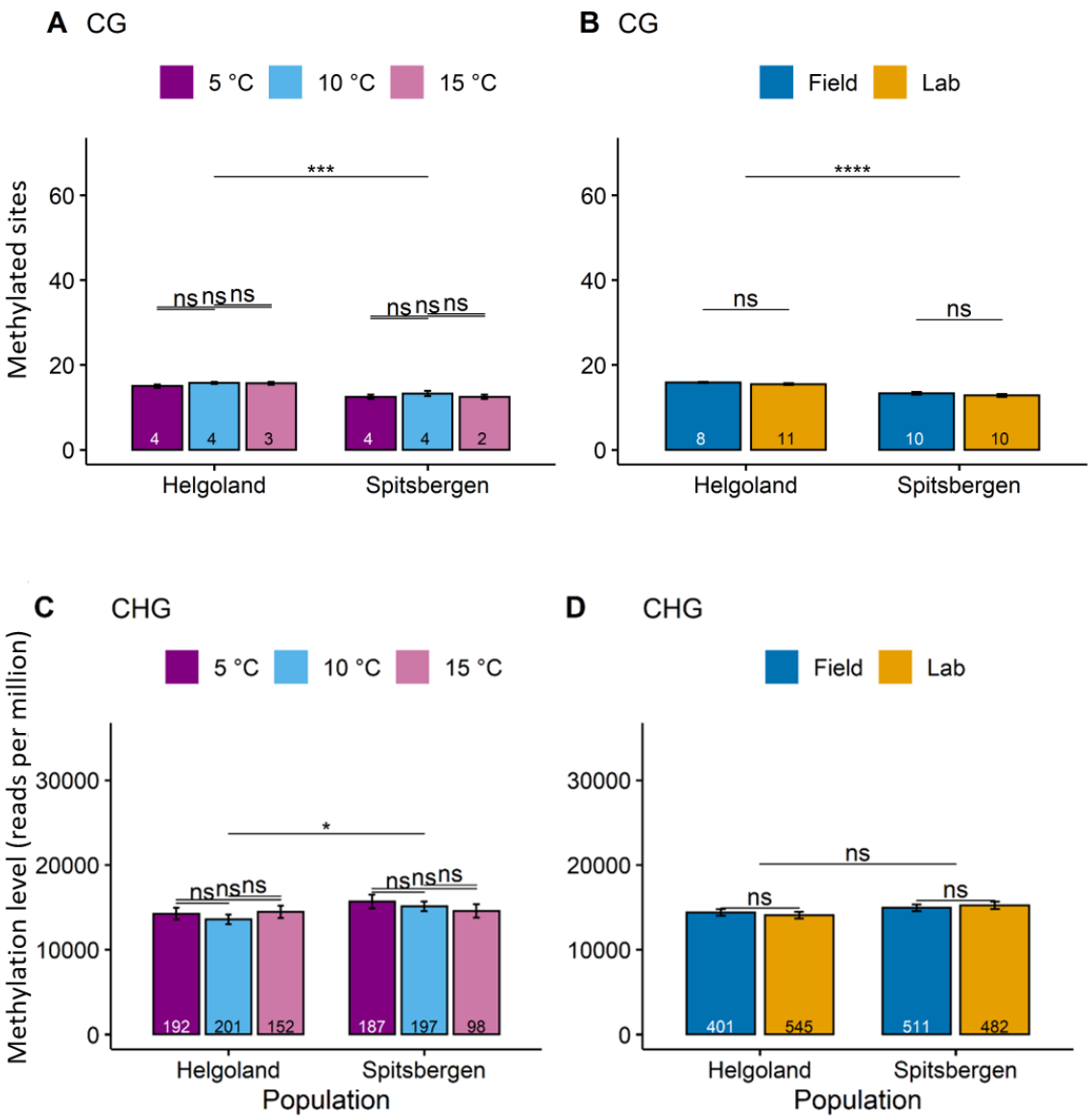
225 Table *1:* Potentially methylated sites for each sequence context, the mean number of methylated sites
226 per sample and standard deviations for the means. Methylation level is the mean percentage of
227 potential methylation sites methylated in samples, shown in the table with standard deviations (SD).

Sequence Context	**Potential Sites (in-silico)**	**Methylated sites (mean +/- SD)**	**Methylation level (mean +/- SD)**
CG	69	14.3 ± 1.5	20.7% ± 2.2%
CHG	201	49.8 ± 3.0	24.8% ± 1.5%

228

229 The number of methylated CG sites was significantly higher in Helgoland than in Spitsbergen
230 samples both in laboratory samples ($p < 0.001$, Fig. 1 A) and in laboratory and field samples
231 combined ($p < 0.0001$, Fig. 1 B), but no significant differences were found between treatment
232 temperatures or between field and laboratory conditions for sites in CHG contexts (Suppl. Fig.
233 3 C, D).

234 Methylation levels normalized to reads per million (RPM) showed no significant differences in
235 CG contexts between any groups (Suppl. Fig. 4 A, B), but the Spitsbergen population showed
236 higher methylation levels than the Helgoland population in the laboratory samples ($p < 0.05$,
237 Fig. 1 C). There were no significant differences between field and lab conditions in CHG context
238 (Fig. 1 D).



239 Figure *1*: **Significant differences in methylated sites and methylation level between conditions.**
240 Methylated CG sites (A, B), and methylation levels as reads per million (RPM) for CHG sites (C, D)
241 between populations and temperatures in laboratory samples (A, C) and compared between lab and
242 field samples (B, D). ns: not significant. Numbers at the bottom of the bars indicate sample size.
243 Significance codes after p-value correction (Benjamini-Hochberg) for multiple comparisons: '< 0.05': *,
244 '< 0.01': **, '< 0.001': ***, '< 0.0001': ****.

Principal Component Analysis

Laboratory and field samples formed distinct groups along the first principal component with 95% confidence ellipses not overlapping when both origins were combined (Fig. 2 A). The first two principal components equally contributed about 14 % of the total sample variation, which was similar between the comparisons of 'lab vs field' (Fig. 2 A) and 'Helgoland vs Spitsbergen' (Fig. 2 B). However, for the origins (Fig. 2 B), the populations of Helgoland and Spitsbergen formed distinct groups along the second principal component when lab and field samples were combined (Fig. 2 B), while when field and laboratory samples were separately analysed for origin, Helgoland and Spitsbergen samples again split along the first component (Fig. 2 C, D). Temperatures in the laboratory samples (5°C, 10°C, 15°C) did not separate into distinct groups (Fig. 2 E).

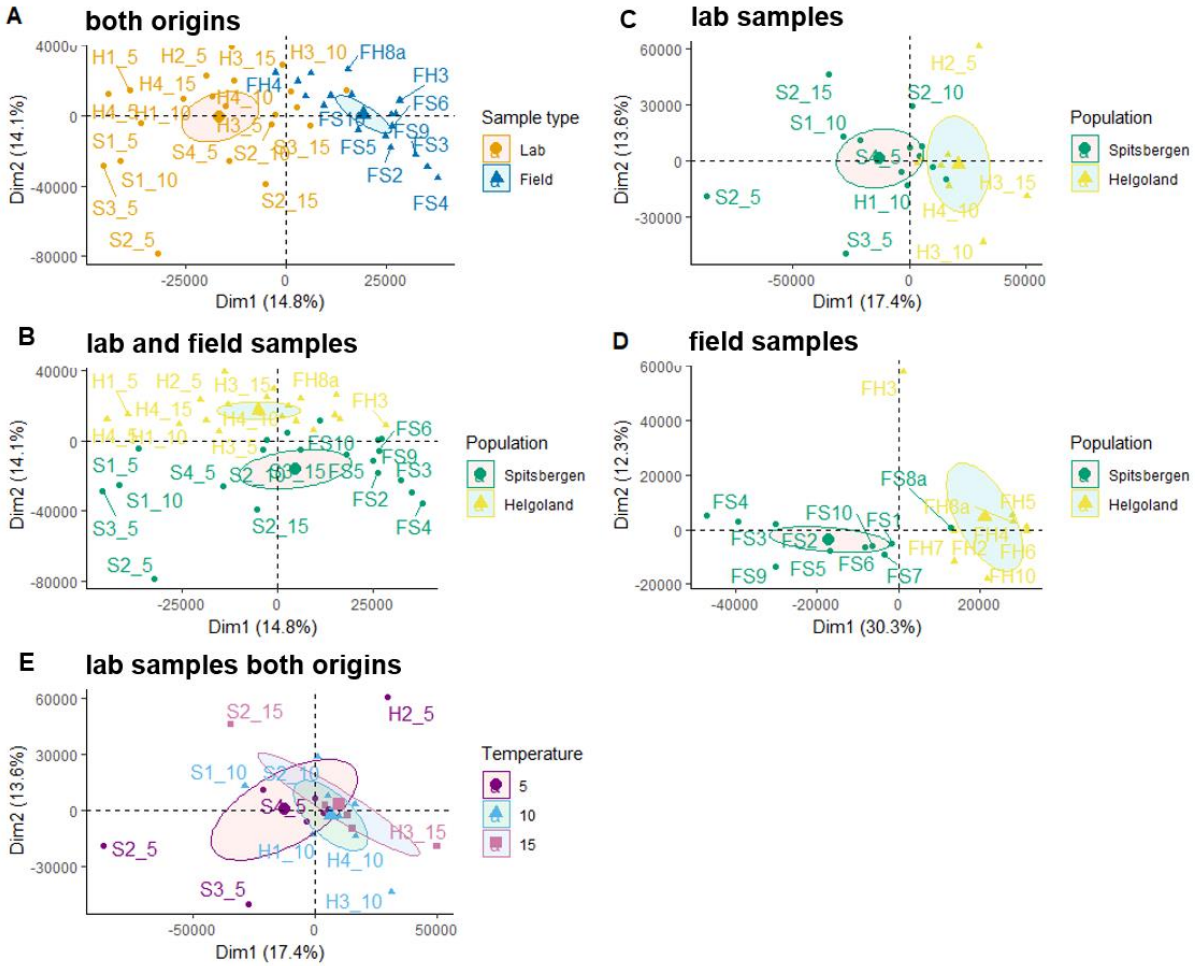


Figure *2*: **Principal Component Analysis for A: all samples, grouped by laboratory or field samples. B: laboratory samples grouped by origin**. C: all samples, grouped by origin. D: field samples grouped by origin. E: laboratory samples of both origins grouped by temperature treatment. Ellipses represent 95% confidence intervals around the group medians.

Differentially methylated sites and GO terms

Comparing the origins (Helgoland vs Spitsbergen, Fig. 3), differentially methylated sites were as often higher methylated in Spitsbergen samples as in Helgoland samples (Suppl.Tab.1-3). Of the eight genes with significant methylation differences between the origins (Fig. 3), only three genes (psaB_(1), atpA, yfc3) were identical to the genes with significant methylation

differences between the origins at a respective temperature within laboratory samples (Table 2, see colour coding suppl. Table 7+8, sheets ‘ST7 Origin’ and ‘ST8 Temperature’).

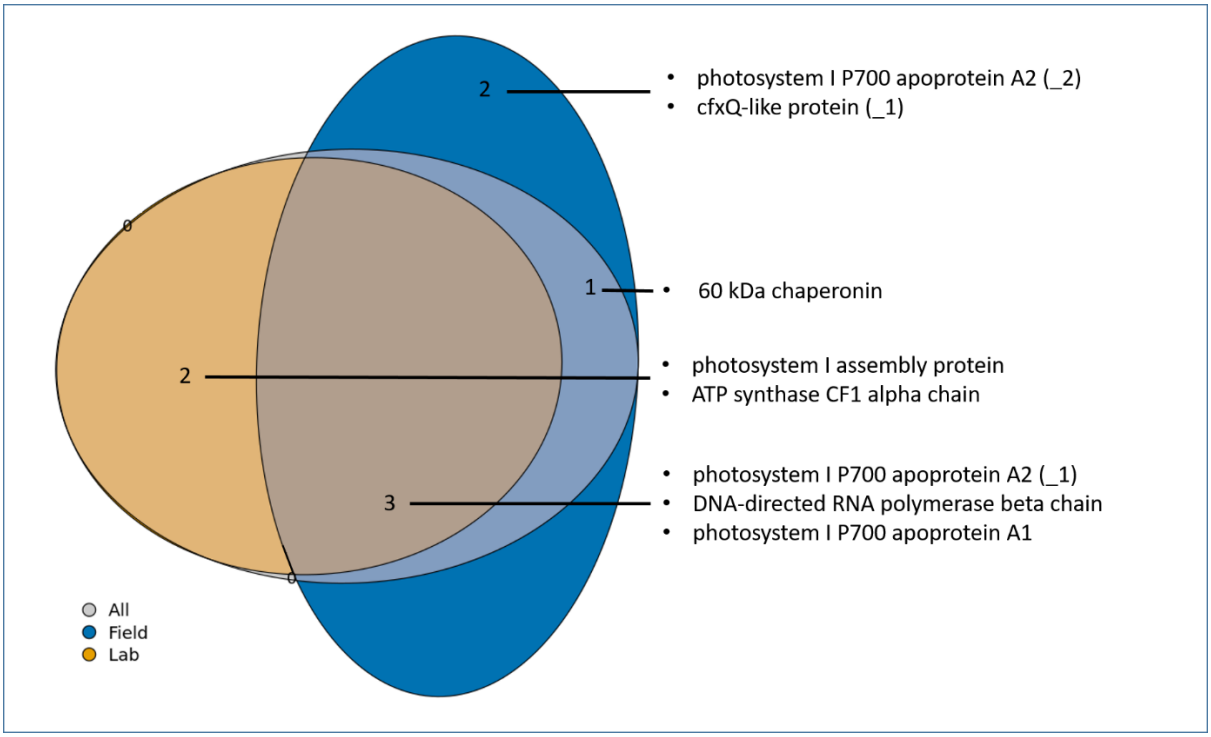
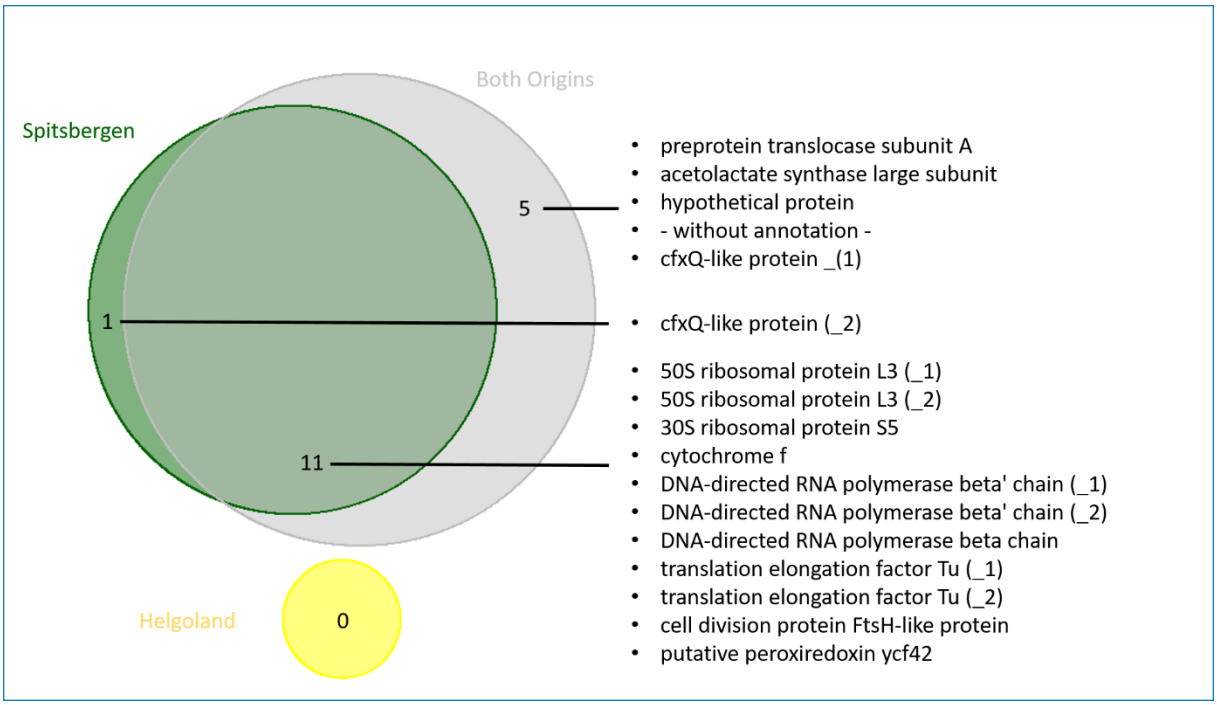


Figure *3:* **VennEuler diagram showing the numbers of differentially methylated sites between Helgoland and Spitsbergen when analysing field samples (blue), lab samples (orange), and all (lab+field, grey) samples** (Suppl. Table 2-4). The labels give the product of the genes that were differentially methylated in the respective partitions. (_1) and (_2) depict a gene that showed differential methylation at two different sites. For the exact site position in the respective gene, see suppl. Table 7 (‘ST7 Origins’).

Of the 69 genes that were analysed comparing growth conditions (Fig. 4), 16 genes were differentially methylated between laboratory and field samples, two of which were also

287 differentially methylated between origins (Fig. 3, Table 2). Many of those genes were

288 differentially methylated at two different positions (suppl. Table 9, sheet 'ST9 Cultivation').



289 Figure *4*: **VennEuler diagram showing the numbers of differentially methylated sites between lab**
290 **and field samples when analysing Helgoland samples (yellow), Spitsbergen samples (green), and the**
291 **samples from both origins together** (**grey**, Suppl. Table 5)**.** The labels give the product of the genes
292 that were differentially methylated in the respective partitions, with one gene without annotation
293 record at the ncbi database. (_1) and (_2) refer to a gene that showed differential methylation at two
294 different positions. For the exact position in the respective gene, see suppl. Table 9 ('ST9 Cultivation').

295

296 Two genes were differentially methylated between temperature treatments (Table 2), with

297 'acetolactate synthase large subunit' (QKE47546.1) at two comparisons: 5°C to 10 °C (padj <

298 0.0001), and 5 °C to 15°C (padj < 0.0017). All other significant differentially methylated genes

299 were found between origins at different temperatures, but only partially overlapped with

300 those obtained when analysing 'all samples' (Fig. 3, see colour codes in suppl. Tables 7 + 8

301 (sheets 'ST7 Origins' + 'ST8 Temperature')).

302

303 Table *2*: **All conditions that were found to be differentially methylated by the DESeq2 analysis when**
304 **analysing the different rearing temperatures of the laboratory samples.** Most significances were
305 detected between the origins at respective temperature (Helgoland vs Spitsbergen @ T5/10/15), but

306 only 3 of those were identical to those detected when all lab samples were being analysed as a group
307 (Fig. 3, Suppl. Table 8 ('ST8 Temperature')). However, genes QKE47546.1 and QKE47431.1 (bold letters)
308 showed significantly different methylation when analysing for temperature difference as factor (origin,
309 T vs T).

Comparison	Gene Product	Gene ID
5° vs 10° Helgoland	**acetolactate synthase large subunit**	**QKE47546.1**
5° vs 15° Helgoland	**acetolactate synthase large subunit**	**QKE47546.1**
5° vs 10° Spitsbergen	**photochlorophyllide reductase subunit B**	**QKE47431.1**
5° Helgoland vs Spitsbergen	photosystem I P700 apoprotein A2 _(1)	QKE47459.1
	ATP synthase CF1 alpha chain	QKE47418.1
	acetolactate synthase large subunit	QKE47546.1
	photochlorophyllide reductase subunit B	QKE47431.1
10° Helgoland vs Spitsbergen	photosystem I P700 apoprotein A2 _(1)	QKE47459.1
	ATP synthase CF1 alpha chain	QKE47546.1
	photosystem I assembly protein	QKE47473.1
	photochlorophyllide reductase subunit B	QKE47431.1
15° Helgoland vs Spitsbergen	photosystem I P700 apoprotein A2 _(1)	QKE47459.1
	ATP synthase CF1 alpha chain	QKE47546.1

310

311 None of the extracted GOterms were significantly enriched according to the 'topGO'
312 enrichment analysis.

313 *Analysis of Epigenetic Distance*

314 In both comparisons ('cultivation', 'origin'), the epigenetic distances between the samples
315 within respective groups were significantly different between groups (Fig. 5). For 'cultivation',
316 distances were significantly higher in laboratory samples than in field samples ($p < 0.0001$,
317 median field 153, lab 210), while for 'origin' the samples obtained from Spitsbergen showed
318 significantly higher epi-distances than Helgoland ($p < 0.0001$, median Helgoland 171,
319 Spitsbergen 190; suppl. Table 6).

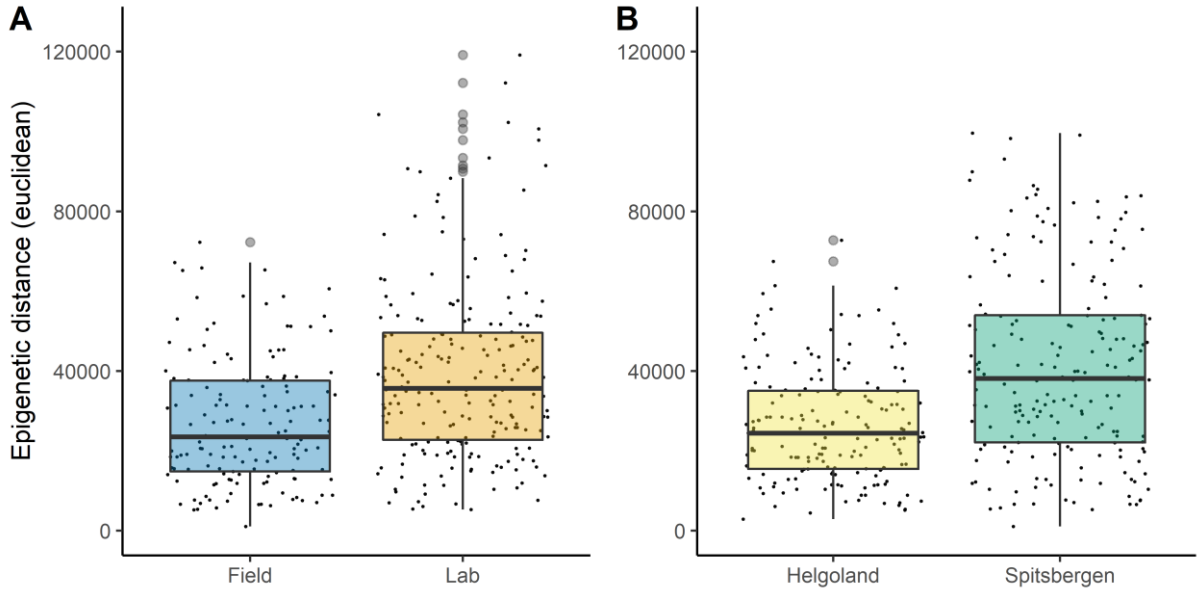


Fig. 5: **Epigenetic distance (Euclidean distance) for 'cultivation', Laboratory (orange) and Field (blue) samples (A), and for 'origin', Helgoland (yellow) and Spitsbergen (green) samples (B).** The small black dots depict the pairwise distances between the individual samples of each group (from the PCA, Fig. 2 A, B). The light grey dots are outliers detected by the boxplot script, but were included during epi-distance analyses. The boxes represent the interquartile range (IQR) of the respective group, and the horizontal line within the boxes gives the median Euclidean distance.

Discussion

Regarding Hypothesis 1, we could confirm a methylome in the chloroplast DNA of *Saccharina latissima* (Table 1). As with the nuclear genome (Scheschonk et al. 2022) the chloroplast methylome significantly differed between origins (Fig. 3, Table 2, Fig. 5 B), and growth conditions (Fig. 4, Fig. 5 A), suggesting a trans-generational epigenetic memory (Hypothesis 2). However, growth temperature had a smaller impact than expected (Table 2; Hypothesis 3). Differentially methylated sites between origins (Spitsbergen or Helgoland) were located in genes with functions related to photosynthesis or ATP synthesis. However, differentially methylated sites between growth conditions (laboratory or field) were predominantly located in genes related to translation and transcription (suppl. Table 7 + 9, sheets 'ST7 Origin' + 'ST9

340 Cultivation'; Fig. 3, Fig. 4). This suggests that the chloroplast methylome is origin-specific,
341 indicating an epigenetic memory, while showing capacity of rapid adaptation to shifts in
342 growth conditions via the epigenetic mechanism of cytosine methylation.

343 *Sample origin*

344 Population origin had a strong impact on the methylome according to PCA clusters (Fig. 2 B,
345 C, D), which was reflected in methylation levels, as well as in the number of methylated sites
346 that significantly differed between origins (Fig 1 A, C). Photosystem I (PSI) was the component
347 most affected by differences between the origins, as genes coding for varying aspects crucial
348 to PSI significantly differed at a total of four sites in three genes (Fig. 3, Table 2), with the
349 strongest effect on PSI P700 chlorophyll a apoprotein A2. The same gene was one of two genes
350 differentially methylated between the origins in all analysed temperature conditions of the
351 laboratory samples (Table 2, suppl. Table 8 sheet 'ST8 Temperature'). Therefore, the
352 difference can be assumed to be solely accounted for by origin. Part of the methylome was
353 observed to be origin-specific even after years of gametophyte lab-cultivation. Methylation
354 levels were significantly different between the origins (Fig. 1), independent from the rearing
355 condition (laboratory or wild). As most of the differentially methylated genes between origins
356 overlapped in field and lab samples (Fig. 3), this indicates the prevalence of a
357 transgenerational epigenetic memory, which is further backed by PCA results. PCA in addition
358 indicated the influence of cultivation on transgenerational memory, as origin played a more
359 pronounced role when analysing laboratory and field samples separately (Fig. 2 C, D) *vs*
360 combined (Fig. 2 B),. The difference was less pronounced in the laboratory than the field
361 samples (Fig. 2 C *vs* D). The differentially methylated genes were exclusively related to
362 functions of photosynthesis (Fig. 3), which likely can be explained by the latitudinal differences
363 in the light regimes between origins. Light conditions in the High-Arctic are strongly seasonal,
364 with absence of photosynthetically active radiation (PAR) for at least three months during the

365 arctic winter (polar night), and continuous PAR during the arctic summer (polar day). In boreal-
366 temperate regions, PAR is available throughout the year. Whether or not the differences
367 observed here are genetically induced cannot be assessed by our setup, but the prevalence of
368 the significant difference, detectable in the epigenetic signature that prevailed between
369 origins through cultivation, is a strong indicator for the eco-genotype approach (Scheschonk
370 et al 2022). This presumes that adaptation to a specific location is not solely a matter of genetic
371 nature (fixed only in the DNA), but that epigenetic mechanisms, especially inheritable ones
372 such as cytosine methylation, play an important role in local adaptation and eco-evolutionary
373 dynamics of a species (Richards and Pigliucci 2020; Jablonka and Lamb 2015) that are partly
374 independent from underlying genetic variation. Considering methylation and gene expression
375 were shown to be negatively correlated in the *S. japonica* nuclear and chloroplast genes (Fan
376 et al. 2020a; Teng et al. 2021) the (double-) methylation of the gene coding for apoprotein A2
377 (Fig. 3, suppl. Table 7 sheet 'ST7 Origin'), for example, which is one of the crucial components
378 of PSI, can be presumed to have a direct impact on the photosynthetic capacity which,
379 according to our results, is likely to differ between origins. Like with the nuclear genome
380 (Scheschonk et al 2022) this has crucial implications for cultivation processes as well as
381 restoration efforts, as it shows the importance of suitable origin when considering local
382 growth condition.

383 *Cultivation effects*

384 PCA showed that cultivation affected the methylome to the same degree as origin (2 A, C).
385 While lab and field samples showed no significant differences in genome-wide methylation
386 level and number of methylated sites (Fig. 1), they displayed significantly different
387 methylation levels for 23.19 % of the methylation sites (Fig. 4, suppl. Table 9 (sheet 'ST9
388 Cultivation'). Most (12 out of 16) of the sites differentially methylated between rearing
389 conditions had higher methylation levels in the lab than in field samples (Suppl. Table 2). This

390 suggests those genes were higher expressed in the field samples (Fan et al. 2020a; Teng et al.
391 2021), possibly due to a higher variety of genes required in the more variable field conditions.
392 Unexpectedly, epigenetic distance was higher in lab samples, even though they experience
393 more uniform environmental conditions than field samples. When translated to transcription,
394 a higher epigenetic distance is likely to result in more variable phenotypes. This might suggest
395 that the kelp methylome diversifies rather under stable lab conditions than under variable
396 field conditions, at least when the given cultivation conditions were not previously
397 encountered. Potential explanatory factors cover the whole process of cultivation, from
398 obtaining the sori, and securing the zoospores, or handling during the fertilisation process, to
399 culture conditions at any stage.

400 However, origin as factor had the strongest influence on the differences in cultivated samples
401 at gene level, while lab-field differences in methylation could only be detected in the
402 Spitsbergen population (Fig. 4, Table 2). A factor that might as well explain differences in the
403 methylomes between lab cultivated and wild kelp is the age of the sporophytes. The field
404 sporophytes were adult, while the lab samples were juveniles with a size of approximately 12-
405 15 cm. In plants, cytosine methylation levels tend to increase with age, but these differences
406 are most pronounced in meristematic areas (Dubrovina and Kiselev 2016), which were
407 avoided in the kelp samples. Because meristematic regions were omitted, and older
408 individuals (field samples) did not show higher methylation levels in the chloroplast genome,
409 the likelihood that the results were affected by sporophyte age differences is low.
410 Temperature as factor could be shown to have an origin-specific impact on the methylome
411 during cultivation (Table 2; see next paragraph). However, which step, or factor, or
412 combination thereof, during the cultivation process is likely to have caused the increase in
413 epigenetic distance will have to be assessed in future studies.

414 Whether or not genotypic differences can result in the epigenetic differences that we
415 observed has yet to be determined. In plants and algae, DNA cytosine methylation can be
416 present in CG, CHG, and CHH sequence contexts, where H is any of the bases A, C, or T
417 (Richards and Pigliucci 2020). Cytosine methylation can be heritable across several
418 generations (Lämke and Bäurle 2017; Anastasiadi et al 2021), hence is a likely candidate for
419 rapid non-genetic but heritable local adaptation. The inheritance of epigenetic traits induced
420 by environmental factors has been repeatedly reported (Foust et al. 2016; Gugger et al. 2016;
421 Keller et al. 2016). The difference in methylation between origins that persisted under
422 laboratory conditions indicates epigenetic inheritance of a molecular memory of the
423 environment. It suggests that methylation differences can remain stable across generations
424 in cultivation, indicating that a transgenerational epigenetic memory likely is present in the
425 *S. latissima* chloroplast genome.

426 *Temperature and the methylome*

427 The temperature treatments did not form distinct clusters in the PCA. Unlike the nuclear
428 methylome, the cultivated *S. latissimas*' chloroplast methylome did not differ in methylation
429 levels and numbers of methylated sites in response to the rearing temperatures of 5 °C, 10 °C
430 and 15 °C. Significant differential methylation solely caused by temperature were detected in
431 2.9 % of the genes (Table 2). However, when analysing the origins at temperature level (Table
432 2), most differences between origins found at discrete temperatures were in other genes than
433 those detected for the origin analysis. This again suggests the inheritance of an epigenetic
434 memory in accordance with the eco-genotype approach (Scheschonk et al. 2022), as the
435 methylomes reacted origin-specific at discrete temperatures. However, it cannot be ruled out
436 whether these epigenetic differences have a genotypic base.

437 *Methylation levels compared with the nuclear genome*

438 The methylation levels of sites in the chloroplast genome were ca. 100 times higher than levels
439 in the nuclear genome as documented by Scheschonk et al. (2022). In the nuclear genome,
440 the mean methylation level was 0.21% ± 0.13% SD (Scheschonk et al. 2022), while in the
441 chloroplast, the levels were 20.7% ± 2.2% for CH contexts and 24.8% ± 1.5% for CHG contexts
442 (Table 1). The higher methylation level in the chloroplast can be explained by the low numbers
443 of potential methylation sites in the chloroplast genome (Table 1). The pattern of higher
444 methylation in the chloroplast than the nuclear genome is repeated in both *S. japonica* and
445 *S. latissima*, with some dissimilarities that could be caused by different sequencing methods
446 and the inclusion of gametophytes in the studies of *S. japonica* (Fan et al. 2020a; Teng et al.
447 2021).

448

449 Photosynthesis is highly sensitive to temperature (Cen and Sage 2005). However, the main
450 temperature response, in terms of methylation, is observable in the nuclear, but not in the
451 chloroplast genome. The lack of statistically significant differences in the chloroplast genome
452 as compared with the nuclear genome might partly be explained by the smaller number of
453 genes. The chloroplast genome has a size of 130 kb and contains 139 protein-coding genes,
454 three rRNA genes and 29 tRNA genes (Fan et al. 2020b), while the nuclear genome has a size
455 of 545 Mb and contains 18,733 protein-coding genes (Ye et al. 2015). However, no significant
456 differences were observed in neither methylation levels nor methylated sites between
457 temperature treatments, despite the ~ 100 x higher methylation level of the chloroplast. In
458 contrast, in the nuclear genome, a response in methylation levels and numbers of methylated
459 sites to warmer temperature treatments was observed, especially in the Helgoland samples
460 (Scheschonk et al. 2022). Possible the lack of statistical significance indicates locations in the

461 methylome where the methylation is highly conserved, and hence crucial on a functional level
462 regardless of origin or external (new) impact. Aother possible explanation for the overall low
463 amount of (differentially) methylated sites might be that the temperature exposure of the
464 laboratory samples was not stressful for the physiological processes regulated by the
465 chloroplast genome. The difference between nuclear and chloroplast methylome suggests
466 that between 5 °C and 15 °C the modulation of temperature responses is solely regulated in
467 the nuclear genome. In *Saccharina latissima*, it has been shown that temperature treatments
468 affect the nuclear methylome, and that differences in the methylome exist between
469 populations from different latitudes, suggesting that epigenetic modifications contribute to
470 local adaptation (Scheschonk et al. 2022). In addition, significant differences in the nuclear
471 methylome were found between wild and cultivated individuals (Scheschonk et al. 2022).
472 Earlier research on *S. latissima* suggests that short-term acclimation to different growth
473 temperatures can lead to changes in heat tolerance, and affect photosynthetic performance
474 (Davison et al. 1991), which supports the temperature priming hypothesis suggested for the
475 nuclear methylome. However, most of the differentially methylated genes were found
476 between origins at discrete temperatures (5/10/15 °C), but were not detected when
477 comparing the origins over all lab samples.

478 In the nuclear genome, most of the differentially methylated sites between lab and field
479 samples and between populations were in non-genic repetitive contexts (transposable
480 elements). In contrast, in the chloroplast genome, the differentially methylated sites were in
481 coding regions of genes, except for very few sites in a promoter region (Suppl. Table 2-5). This
482 is not surprising, given that chloroplast genomes are more condensed and have far fewer
483 noncoding regions than nuclear DNA (Clegg et al. 1994).genomes.

484 *Conclusion*

485 The chloroplast genome mainly contributes to processes of photosynthesis. Our results
486 showed that sample origin and cultivation can strongly influence epigenetic mechanisms in
487 the chloroplast, while temperature had a minor effect. Eco-phenotypes are likely developed
488 in the chloroplast, as methylation differences in genes are prone to trigger differences in gene
489 expression (Richards and Pigliucci 2020, Anastasiadi et al 2021). The scale-up in cultivation of
490 *Saccharina latissima* during the last decade is likely to continue, and cultivation sites overall
491 will likely shift northward, in accordance to the preferences of wild populations. The capacity
492 of fast adaptation to changed environmental factors, facilitated by epigenetic mechanisms,
493 could prove particularly useful in the face of rapid anthropogenic climate change. However,
494 due to the high ecological and economic value of this kelp, the implication of changes in
495 methylation due to cultivation, and especially the possibility of differential chloroplast
496 methylation influencing photosynthetic capacity, need to be further assessed. Photosynthetic
497 capacity is directly linked to primary production, and the capability to build up and maintain
498 biomass. Processes impacting the chloroplast methylome would have implications for both
499 cultivated and wild populations. In cultivars, the importance of matching hatchery and
500 cultivation conditions to origin has been implied by our results. Furthermore, due to the
501 significant differences observed in the methylomes of cultivated *S. latissima*, it might be
502 possible to detect an epigenetic 'fingerprint' of cultivated specimen in the future, to detect
503 escapees to the wild. Regarding the current trend in northward shift, the further north
504 populations of *Saccharina latissima* settle, the more pressure the months of darkness during
505 polar night will pose (Scheschonk et al. 2019). Photosynthetic pigments were shown to be
506 functional throughout the prolonged darkness of the Arctic winter (Scheschonk et al 2019),
507 indicating the chloroplasts of High Arctic specimen retained photosynthetic capacity during
508 polar night. In which context differential methylation of chloroplast DNA facilitated by

509 differences in the eco-phenotypes plays a role in this context will be of interest, especially
510 regarding populations adapted to light conditions at more southern distributional habitats,
511 shifting northwards. In *Saccharina latissima*, phenotypic variation among populations is often
512 driven by phenotypic plasticity, and the species shows high plasticity in response to changing
513 abiotic factors (Bolton & Lüning 1982; Diehl and Bischof 2021). So far, research on differences
514 between origins in *S. latissima* has focused on genetic differentiation, with significant genetic
515 variation between populations (Møller Nielsen et al. 2016; Paulino et al. 2016; Guzinski et al.
516 2020). Epigenetic differences have largely been unexplored as an alternative source of
517 molecular variation between *S. latissima* populations. The differences we observed between
518 origins within the laboratory and the field samples indicate that the eco-phenotype approach
519 (Scheschonk et al 2021) is applicable to the organellar chloroplast genome as well.
520 Furthermore, our data suggests that the influence of cultivation seems to be at least partially
521 origin-specific, or that the influence of cultivation and origin on the methylome are
522 interconnected. Epigenetic modifications have been found to be under some degree of
523 genetic control, but can also be triggered by environmental factors such as temperature
524 changes (Boyko et al. 2010; Correia et al. 2013). Hence, the role of genetic differences
525 controlling the epigenetic responses reported here needs to be assessed in further detail in
526 future studies.

527

528 ## Data availability

529 Raw sequencing data of this study are available at NCBI SRA BioProject PRJNA809008.

530

Acknowledgements

We thank S. Niedzwiedz (University of Bremen, Germany) for securing the field samples, and G. Hoarau and H. Reiss (Nord University, Bodø, Norway) for providing lab space and sequencer. Thank you, M. Kopp (Nord University, Bodø, Norway), for the support during the sequencing procedure, and for preparing some of the libraries. Thanks to I. Bartsch and A. Wagner (Alfred Wegener Institute for Marine and Polar Research, Bremerhaven, Germany) for providing and hatching the sporophyte cultures.

Funding

LS acknowledges funding by Deutscher Akademischer Auslandsdienst (DAAD; research travel grant 'BremenIdeaOut' 2019). AN acknowledges funding by Nord University (Master Thesis and transitional stipend). AJ acknowledges funding by Nord University (research talents grant). Parts of this work have been submitted in partial fulfilment of the requirements for Master of Science, at Nord University, Bodo (AN), and Dr. rer. nat, at University of Bremen (LS).

Author Contributions

LS designed and conducted the experiment. AN, LS and AJ analysed and interpreted the data. LS and AN wrote the manuscript. All authors read and commented on previous versions of the manuscript, and approved the final version.

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715 Figure Legends

716 Figure *1*: **Significant differences in methylated sites and methylation level between conditions.**
717 Methylated CG sites (A, B), and methylation levels as reads per million (RPM) for CHG sites (C, D)
718 between populations and temperatures in laboratory samples (A, C) and compared between lab and
719 field samples (B, D). ns: not significant. Numbers at the bottom of the bars indicate sample size.
720 Significance codes after p-value correction (Benjamini-Hochberg) for multiple comparisons: '< 0.05': *,
721 '< 0.01': **, '< 0.001': ***, '< 0.0001': ****.

722

723 Figure *2*: **Principal Component Analysis for A: all samples, grouped by laboratory or field samples. B:**
724 **laboratory samples grouped by origin**. C: all samples, grouped by origin. D: field samples grouped by
725 origin. E: laboratory samples of both origins grouped by temperature treatment. Ellipses represent
726 95% confidence intervals around the group medians.

727

728 Figure *3:* **VennEuler diagram showing the numbers of differentially methylated sites between**
729 **Helgoland and Spitsbergen when analysing field samples (blue), lab samples (orange), and all**
730 **(lab+field, grey) samples** (Suppl. Table 2-4). The labels give the product of the genes that were
731 differentially methylated in the respective partitions. (_1) and (_2) depict a gene that showed
732 differential methylation at two different sites. For the exact site position in the respective gene, see
733 suppl. Table 7 ('ST7 Origins').

734

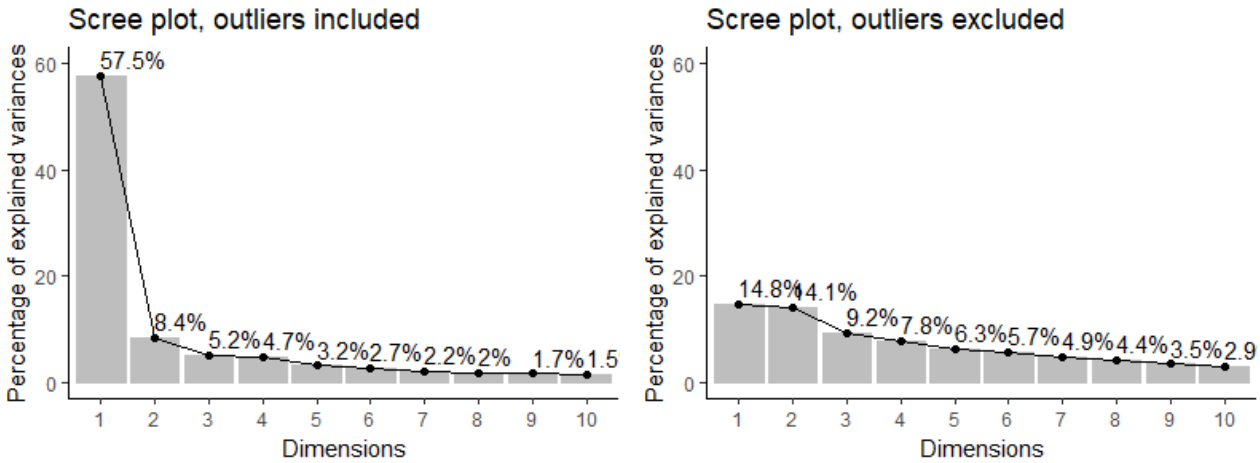
735 Figure *4*: **VennEuler diagram showing the numbers of differentially methylated sites between lab**
736 **and field samples when analysing Helgoland samples (yellow), Spitsbergen samples (green), and the**

samples from both origins together (**grey**, Suppl. Table 5)**.** The labels give the product of the genes that were differentially methylated in the respective partitions, with one gene without annotation record at the ncbi database. (_1) and (_2) refer to a gene that showed differential methylation at two different positions. For the exact position in the respective gene, see suppl. Table 9 ('ST9 Cultivation').

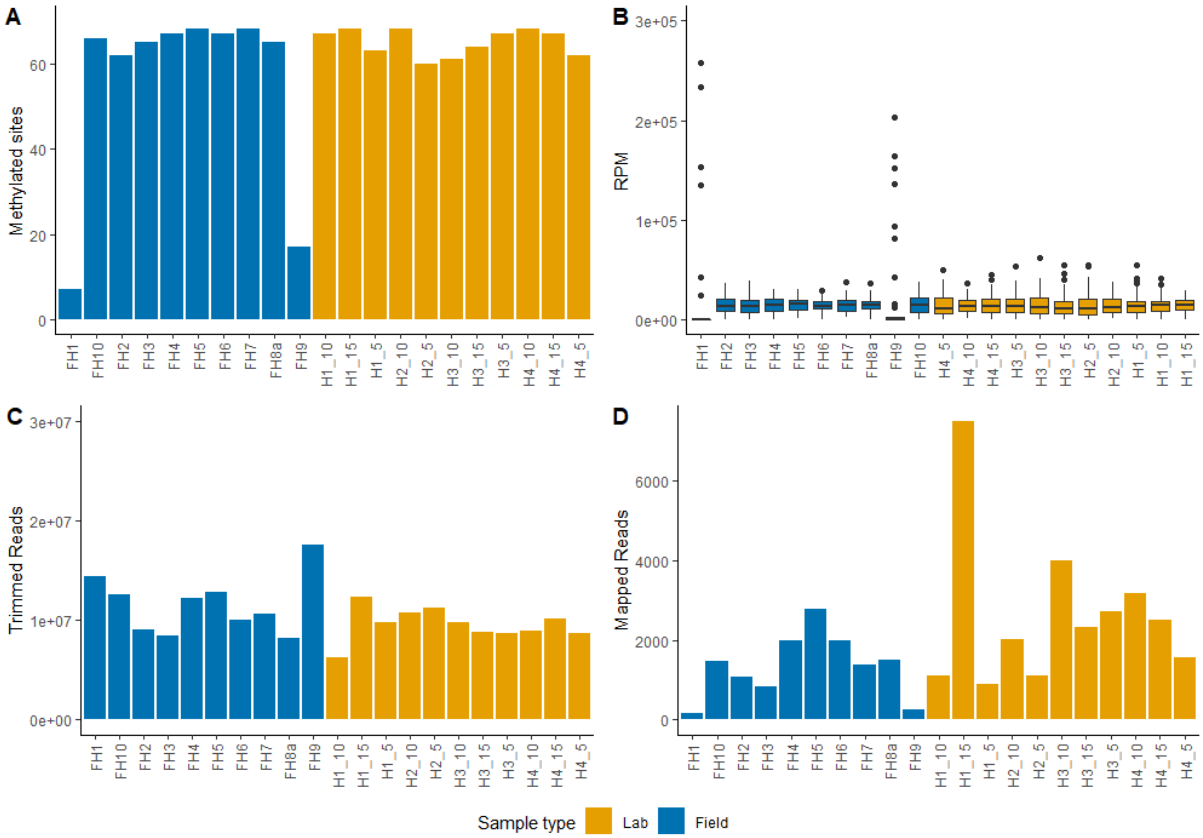
Fig. 5: **Epigenetic distance (Euclidean distance) for 'cultivation', Laboratory (orange) and Field (blue) samples (A), and for 'origin', Helgoland (yellow) and Spitsbergen (green) samples (B).** The small black dots depict the pairwise distances between the individual samples of each group (from the PCA, Fig. 2 A,B). The light grey dots are outliers detected by the boxplot script, but were included during epi-distance analyses. The boxes represent the interquartile range (IQR) of the respective group, and the horizontal line within the boxes gives the median Euclidean distance.

Supplementary

Suppl. Figures

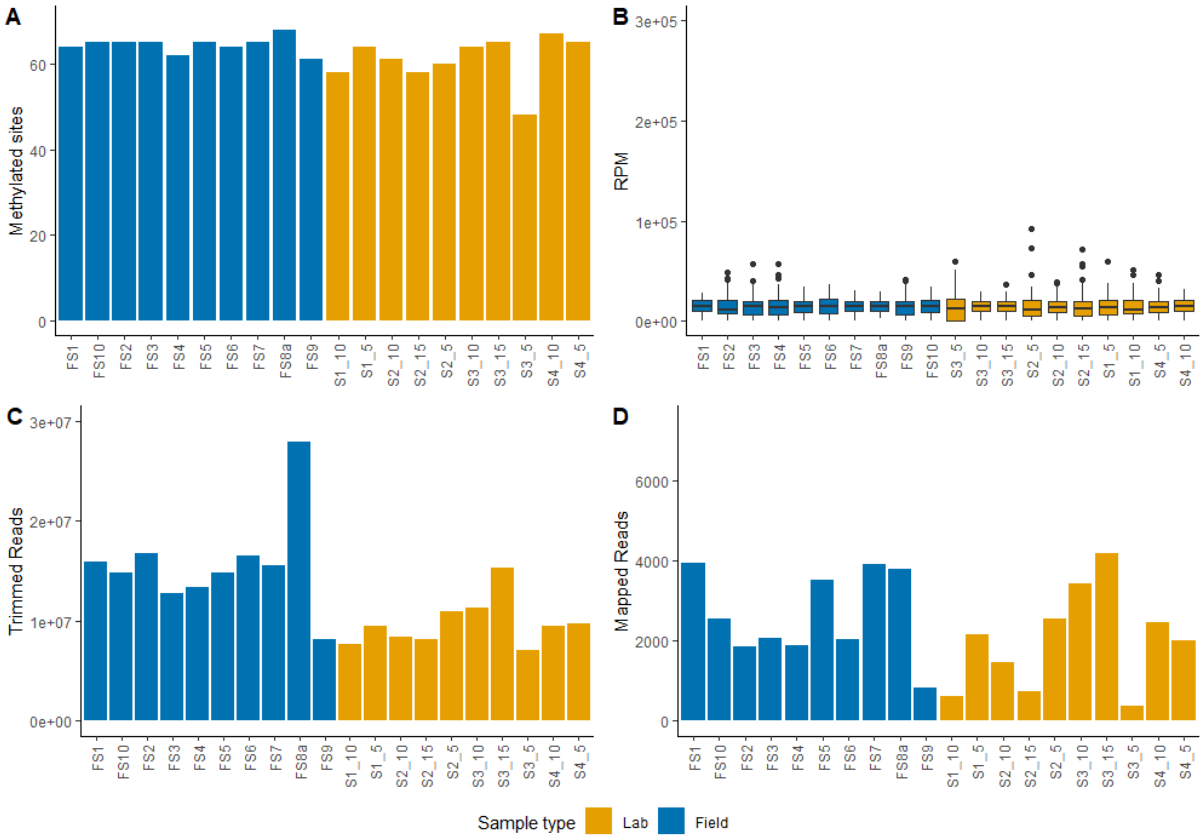


Suppl. Fig. *1*: **Scree plots showing the amount of variance explained by each dimension**. On the left including all samples, on the right when removing two outliers.



759 Suppl.Fig. *2*: **Sequencing output and methylation characteristics for each sample from Helgoland.**
760 Plots were used for outlier detection for PCA. A: Number of methylated sites (with more than two
761 reads) for each sample. B: Boxplot showing reads per million (RPM) for each sample, with each point
762 representing one methylated site. C: The number of sequenced and quality-trimmed reads for each
763 sample. D: Numbers of quality-trimmed reads that mapped back to the chloroplast genome of
764 *Saccharina latissima*.

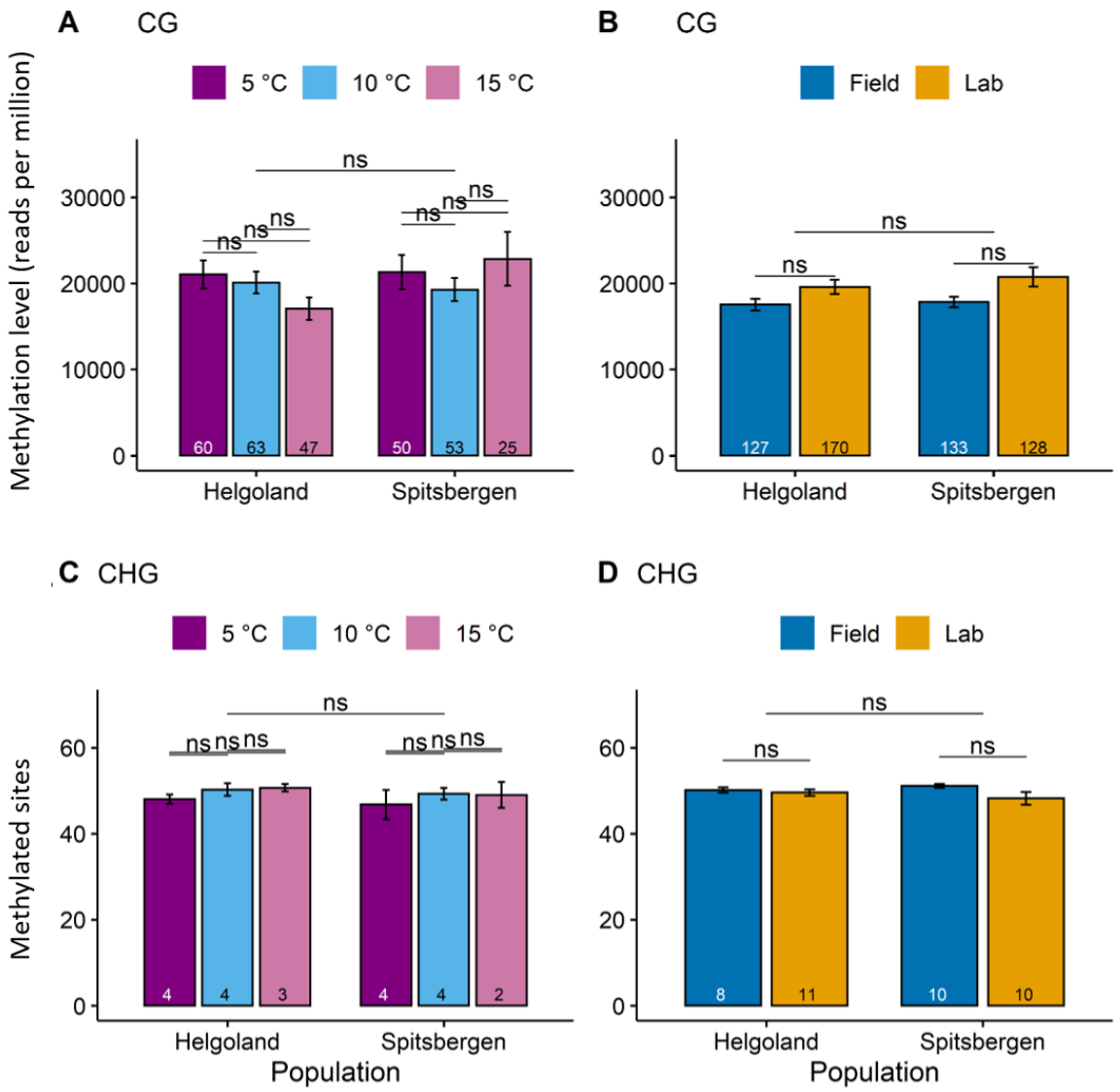
765



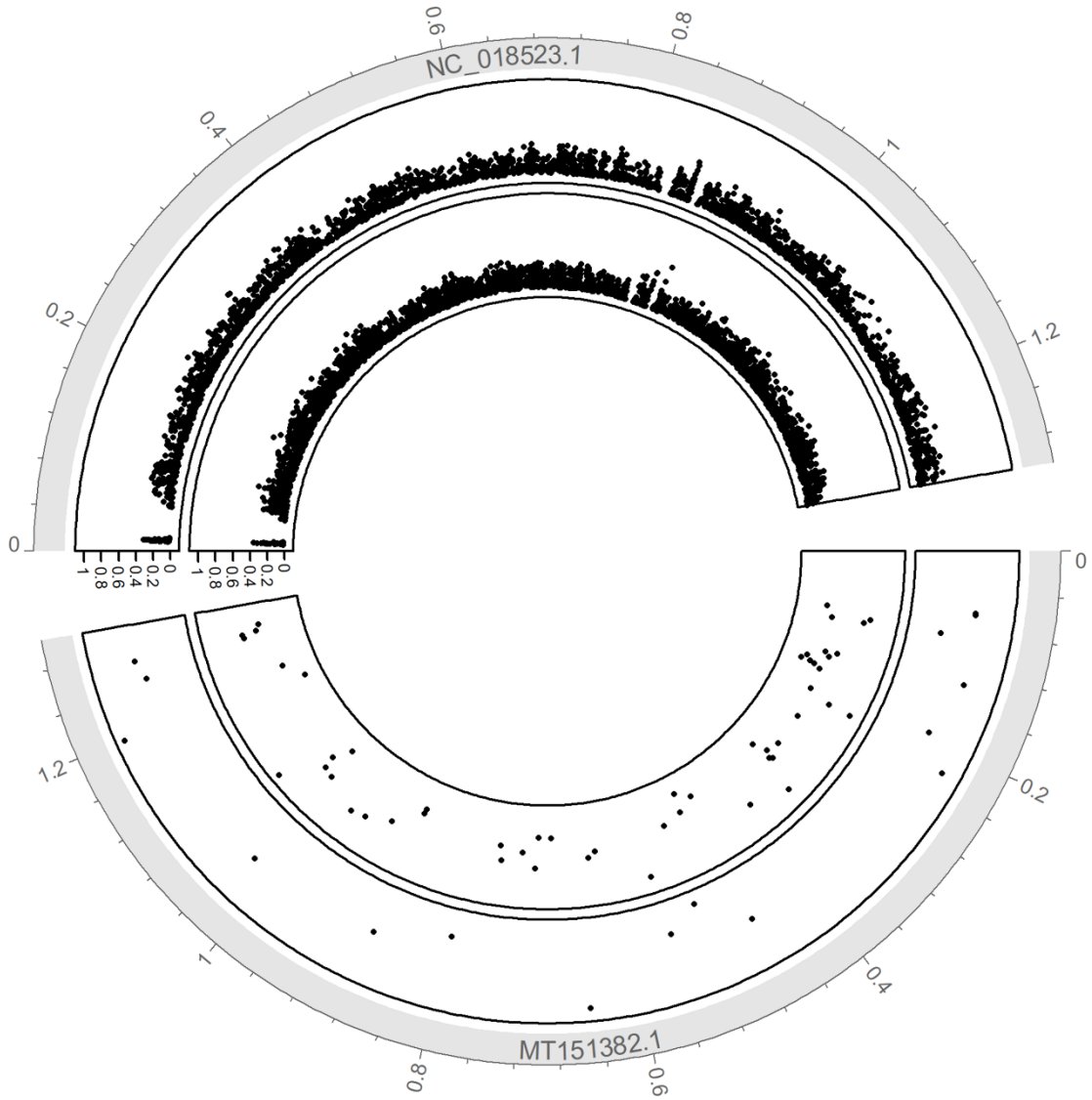
766

767 Suppl.Fig. *3*: **Sequencing output and methylation characteristics for each sample from Spitsbergen.**
768 Plots were used for outlier detection for PCA. A: Number of methylated sites (with more than two
769 reads) for each sample. B: Boxplot showing reads per million (RPM) for each sample, with each point
770 representing one methylated site. C: The number of sequenced and quality-trimmed reads for each
771 sample. D: Numbers of quality-trimmed reads that mapped back to the chloroplast genome of
772 *Saccharina latissima*.

773



Suppl. Fig. *4*: D**ifferences in methylated sites and methylation level between conditions.** Methylation levels as reads per million (RPM) for CHG sites (A, B), and methylated CG sites (A, B) between populations and temperatures in laboratory samples (A, C) and compared between lab and field samples (B, D). ns: not significant. Numbers at the bottom of the bars indicate sample size.



Suppl.Fig. *5:* **Overview of the chloroplast methylome in *S. latissima* (MT151382) and *S. Japonica* (NC_0198523.1).** *S. japonica* data from Teng et al. 2021. Numbers on the outside of the circle represent locations within the genomes. Numbers on y-axes show the methylation levels at each site. The inner circle shows CHG methylation, and the outer circle shows CG methylation. Numbers along the outer circles refer to megabases along the chloroplast genomes.

Suppl. Table 1: Fertilisation scheme of the laboratory male (♂) and female (♀) gametophyte cultures from Helgoland (HG1-4) and Spitsbergen (SG1-4), resulting in the cultures (Helgoland (H1-4) and Spitsbergen (S1-4) raised under laboratory conditions.

Gametophyte Cultures	HG2 ♀ X HG2 ♂	HG3 ♀ X HG3 ♂	HG4 ♀ X HG4 ♂	SG1 ♀ X SG1 ♂	SG2 ♀ X SG2 ♂	SG3 ♀ X SG3 ♂	SG34 ♀ X SG4 ♂
Sporophyte Culture	H2	H3	H4	S1	S2	S3	S4

Suppl. Table 2 Sites differentially methylated (p < 0.05) between Helgoland and Spitsbergen populations when including all samples in the comparison. Positive Log2FC values indicate increased methylation in Spitsbergen compared to Helgoland, while negative values indicate higher methylation in Helgoland samples.

Location	Log2FC	Log2FC std error	Adjusted p value	Gene ID	Gene name	Region	Product	Sequence context
21922-21953	1.04	0.196	2.66E-06	QKE47428.1	rpoB	gene	DNA-directed RNA polymerase beta chain	CHG
68544-68575	0.933	0.302	0.0274	QKE47473.1	ycf3	gene	photosystem I assembly protein	CHG
39358-39389	0.456	0.159	0.0475	QKE47440.1	groEL	gene	60 kDa chaperonin	CHG
51728-51760	-4.00	0.903	0.000164	QKE47458.1	psaA	promoter	photosystem I P700 apoprotein A1	CG
9045-9077	-4.17	0.77	2.05E-06	QKE47418.1	atpA	gene	ATP synthase CF1 alpha chain	CG
55344-55376	-4.96	0.644	9.14E-13	QKE47459.1	psaB	gene	photosystem I P700 apoprotein	CG

							A2	

Suppl. Table 3

Sites with differential DNA methylation ($p < 0.05$) between Helgoland and Spitsbergen populations when comparing only field samples. Positive Log2FC values indicate increased methylation in Spitsbergen compared to Helgoland, while negative values indicate higher methylation in Helgoland samples.

Location	Log2FC	Log2FC std error	Adjusted p value	Gene ID	Gene name	Region	Product	Sequence context
21922-21953	0.867	0.256	0.0117	QKE47428.1	rpoB	gene	DNA-directed RNA polymerase beta chain	CHG
39358-39389	0.776	0.222	0.0104	QKE47440.1	groEL	gene	60 kDa chaperonin	CHG
75619-75650	0.727	0.206	0.0104	QKE47479.1	cbbX	gene	cfxQ-like protein	CHG
55567-55598	-0.528	0.173	0.0259	QKE47459.1	psaB	gene	photosystem I P700 apoprotein A2	CHG
51728-51760	-3.15	0.994	0.0209	QKE47458.1	psaA	promoter	photosystem I P700 apoprotein A1	CG
55344-55376	-4.49	1.21	0.0104	QKE47459.1	psaB	gene	photosystem I P700 apoprotein A2	CG

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794

795

Suppl. Table 4

Sites with differential DNA methylation (p < 0.05) between samples originating from Helgoland or Spitsbergen when comparing only laboratory samples. Positive Log2FC values indicate increased methylation in Spitsbergen compared to Helgoland, while negative values indicate higher methylation in Helgoland samples.

Location	Log2FC	Log2FC std error	Adjusted p value	Gene ID	Gene name	Region	Product	Sequence context
68544-68575	1.43	0.457	0.023	QKE47473.1	ycf3	gene	photosystem I assembly protein	CHG
21922-21953	1.18	0.285	0.000824	QKE47428.1	rpoB	gene	DNA-directed RNA polymerase beta chain	CHG
9045-9077	-5.24	0.701	2.71E-12	QKE47418.1	atpA	gene	ATP synthase CF1 alpha chain	CG
51728-51760	-5.24	1.43	0.00438	QKE47458.1	psaA	promoter	photosystem I P700 apoprotein A1	CG
55344-55376	-5.68	0.639	4.27E-17	QKE47459.1	psaB	gene	photosystem I P700 apoprotein A2	CG

796

797

Suppl. Table 5

Sites differentially methylated (p < 0.05) between laboratory and field samples. Positive Log2FC values indicate higher methylation in laboratory samples compared to field samples.

Location	Log2FC	Log2FC std error	Adjusted p value	Gene ID	Gene name	Region	Product	Sequence context
88361-88392	2.27	0.479	0.000137	QKE47493.1	rpl3	gene	50S ribosomal protein L3	CHG
15235-15266	1.16	0.282	0.000659	QKE47426.1	rpoC2	gene	DNA-directed RNA polymerase beta' chain	CHG
88373-88404	0.987	0.298	0.00741	QKE47493.1	rpl3	gene	50S ribosomal protein L3	CHG
17309-17340	0.952	0.31	0.0124	QKE47426.1	rpoC2	gene	DNA-directed RNA polymerase beta' chain	CHG
64927-64959	0.752	0.173	0.000294	QKE47469.1	petA	gene	cytochrome f	CG
34231-34262	0.598	0.198	0.0134	QKE47436.1	secA	promoter	preprotein translocase subunit A	CHG
102711-102742	0.591	0.172	0.00654	QKE47520.1	tufA	gene	translation elongation factor Tu	CHG
124685-124717	0.561	0.181	0.0123	QKE47544.1	ycf39	gene	NmrA domain-containing protein	CG
46721-46753	0.551	0.195	0.0237	QKE47451.1	ycf42	gene	putative peroxiredoxin ycf42	CG
32980-33011	0.514	0.143	0.00416	QKE47436.1	secA	gene	preprotein translocase subunit A	CHG
102611-102643	0.49	0.175	0.0241	QKE47520.1	tufA	gene	translation elongation factor Tu	CG
22542-22574	0.439	0.161	0.0264	QKE47428.1	rpoB	gene	DNA-directed RNA polymerase beta chain	CG
75988-76019	-0.435	0.158	0.0253	QKE47479.1	cbbX	gene	cfxQ-like protein	CHG
104220-104251	-0.567	0.173	0.00741	QKE47523.1	ftsH	gene	ATP-dependent zinc metalloprotease FtsH	CHG
127333-127364	-0.641	0.191	0.00741	QKE47546.1	ilvB	gene	acetolactate synthase large subunit	CHG
95574-95605	-0.728	0.16	0.000183	QKE47509.1	rps5	gene	30S ribosomal protein S5	CHG

Suppl.Table *6:* Statistics results of the epigenetic distance analysis.					
Epidistance field/lab test results				**Euclidean distance**	
Wilcoxon rank sum test		*statistic*	10499		**Mean dist.**
		p	0.0000000173	*Field*	153
		p.signif	****	*Lab*	219
Shapiro-Wilks test	*Field*	*statistic (W)*	0.945		
		p	0.00000106		
	Lab	*statistic (W)*	0.932		
		p	0.0000000264		
Epidistance Helgoland/Spitsbergen test results				**Euclidean distance**	
Wilcoxon rank sum test		*statistic*	10330		**Mean dist.**
		p	0.00000000231	*Helgoland*	171
		p.signif	****	*Spitsbergen*	190
Shapiro-Wilks test	*Helgoland*	*statistic (W)*	0.965		
		p	0.0000305		
	Spitsbergen	*statistic (W)*	0.957		
		p	0.0000172		

Suppl.Table ***7*** see excel file 'SupplTab7_8_9_Fig3_4_Tab2' sheet 'ST7 Origins'
Suppl.Table ***8*** see excel file ' SupplTab7_8_9_Fig3_4_Tab2' sheet 'ST8 Temperature'
Suppl.Table *9* see excel file ' SupplTab7_8_9_Fig3_4_Tab2' sheet 'ST9 Cultivation'