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Seasonal and spatial variations in biomarker response within Arctic populations of mussels (*Mytilus* spp.)

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Keywords: environmental monitoring, reproductive cycle, bivalve, mollusc, littoral zone, Norwegian Sea

Highlights:

- The study provides unique data on wild *Mytilus* spp. from the North Norwegian coast
- Spawning was partial during summer when water temperature ranged from 10 to 14 °C
- Contaminant levels accumulated in mussels significantly increased from 2010 to 2014
- Maturity phase and tidal zone had the strongest influence on baseline biomarker levels
- Neutral red retention assay has to be adjusted to mussel ambient temperature

Running title: Baseline biomarkers in Arctic *Mytilus* spp.

31 Abstract
32 Climate change and pollution resulting from human activities in the Arctic require
33 reliable monitoring systems and these are usually based on biomarkers in sentinel species.
34 *Mytilus* spp. are used as sentinel species all around the world. The use of *Mytilus* spp. in
35 environmental monitoring requires knowledge of their biology and natural variations in
36 biomarkers. Seasonal variations in baseline levels of pollution biomarkers were studied
37 over a year in the mussels from upper and lower littoral zone in Rakkfjorden, Norway, as
38 they underwent their annual reproductive cycle. Spatial variations of these baseline
39 biomarker levels were measured in five mussel populations within a 60-km radius from
40 Rakkfjorden to investigate universality of the results from the specific population of
41 Rakkfjorden at a regional scale. Seasonal variations in biomarker levels were revealed
42 and seemed to be related to the reproductive state of the mussels and the tidal zone. The
43 mussels appeared to be more sensitive to oxidative stress during gametogenesis in
44 autumn and winter, when having lower lysosome membrane stability and lower baseline
45 levels of antioxidant biomarkers. An increase in baseline levels of these biomarkers was
46 reported during spawning in spring, however, it was not possible to reveal whether these
47 changes were due to spawning, or to a higher metabolic activity in mussels in response to
48 elevated water temperature and food abundance. The differences between the tidal zones
49 reflected in reduced size of the mussels from the upper littoral zone, their late spawning
50 in the season and increased baseline levels of antioxidant biomarkers during the coldest
51 month, indicating a more challenging environment in the upper littoral zone. The spatial
52 study indicated that the baseline biomarker levels measured in Rakkfjorden were no
53 different from the levels measured in the mussels from five other sites and thus, are
54 representative for all mussels on a regional scale.

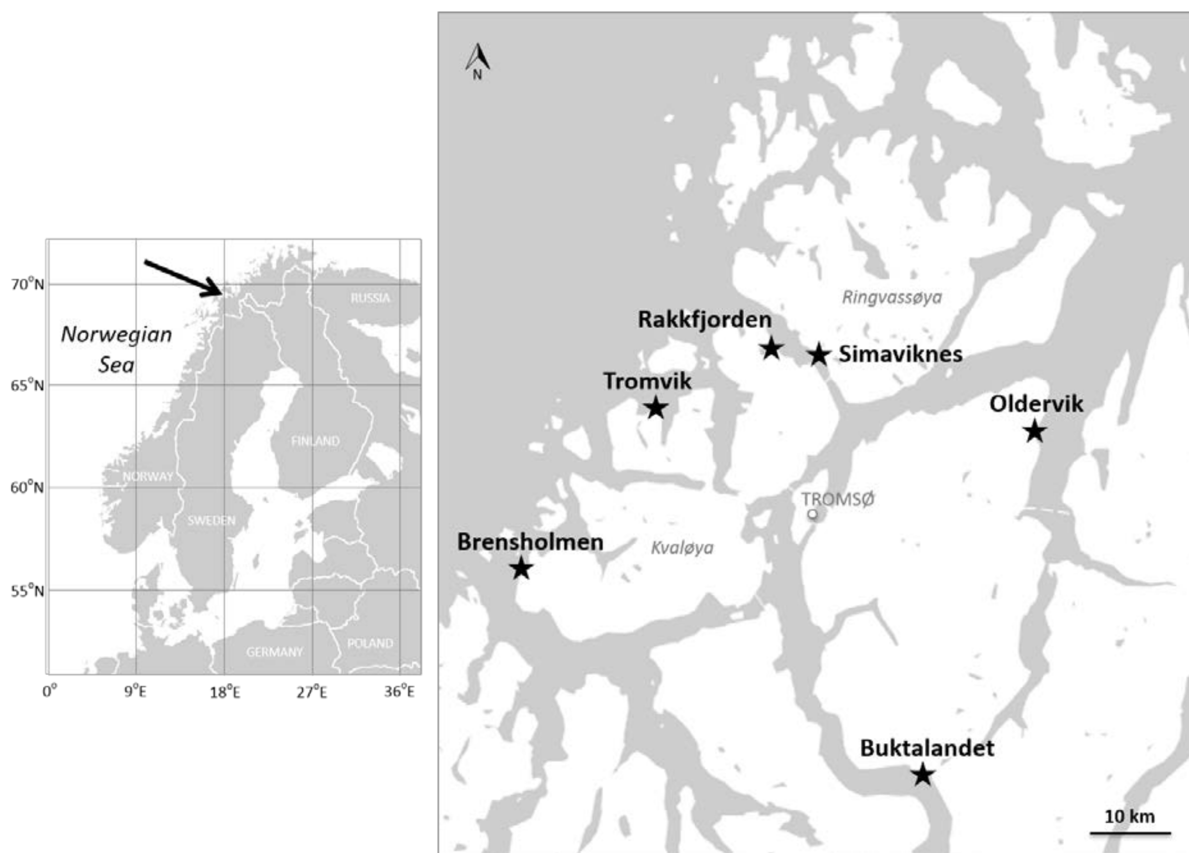
1. Introduction

Bivalves, such as mussels (family *Mytilidae*), are usually used as sentinel species in environmental monitoring (Caza et al., 2016; Azizi et al., 2018). They are filter feeders that may absorb and accumulate pollutants in their tissues. They are sessile, so the pollutants accumulated are representative of a specific location. Mussels (*Mytilus* spp.) are used as sentinel species in many parts of the world, both in large environmental monitoring programmes, such as the Mussel Watch Programme in the USA (Goldberg, 1986) and at more local scales (Laffon et al., 2006). For example, in Norway, the blue mussel (*Mytilus edulis*) is used to monitor effects of offshore petroleum activities (Hylland et al., 2008; Brooks et al., 2011).

Increases in oil and gas activities (AMAP, 2010; Harsem et al., 2011), shipping (Smith and Stephenson, 2013) and tourism (Arneberg et al., 2009; Dybedal et al., 2015) in northern regions create increased risks for acute and chronic pollution. Arctic marine environments, especially coastal habitats are considered to be sensitive areas with high biodiversity, unique ecology and economical value. Biomarkers are commonly used as early warning tools in environmental monitoring (Cajaraville et al., 2000), allowing to detect alterations at various levels of biological organization in response to pollutant and other possible environmental stressors. Prior to adopting biomarkers for environmental monitoring it is important to have knowledge about their baseline and natural variations (van der Oost et al., 2003), which can be affected by seasonal variations related to cycles of feeding, growth and reproduction (González-Fernández et al., 2016). Also, the baseline biomarker levels can vary due to spatial differences related to local variations in the environmental conditions including within a mussel bed between lower and upper littoral zone (Petes et al., 2007), and on a larger spatial scale, variations in oceanographic regime (Pfeifer et al., 2005; Kamel et al., 2014; Vidal-Liñán, 2015) and pollution (Gagne et al., 2008).

In the present study, baseline levels of biomarkers of oxidative stress (lipid peroxidation, catalase [CAT] activity, glutathione S-transferase [GST] activity, total oxyradical scavenging capacity [TOSC]), and lysosome membrane stability (LMS) were studied. These biomarkers are used in most monitoring programmes and reported in the International Council for the Exploration of the Sea (ICES) guidelines (ICES, 2010). The main objective was to investigate seasonal changes and spatial differences in these biomarkers in mussels *Mytilus* spp. from

86 Arctic coastal sites and discuss them in relation to exogenous (season, water temperature, tidal
87 zone, phytoplankton abundance, pollutants) and endogenous (size, gender, reproductive stage)
88 factors. In the seasonal study, the baseline variations of biomarkers were studied over a year in
89 mussels from upper and lower littoral zones in Rakkfjorden, Kvalsundet, Troms County,
90 Norway (69°49´N 18°55´E, Fig. 1). We hypothesized that the levels of biomarkers vary over
91 the course of a year due to changes in biological function associated with the annual
92 reproductive cycle (Schmidt et al., 2013; González-Fernández et al., 2016) and seasonality in
93 environmental conditions (photoperiod, temperature, salinity and food supply) (Bebianno et al.,
94 2007; Brenner et al., 2014). Moreover, differences in biomarkers related to the location of the
95 mussels within the mussel bed were expected (Letendre et al., 2009; Schmidt et al., 2012).
96 Exposure to harsher environmental conditions (e.g. desiccation, light, and temperature) in the
97 mussels on the upper littoral zone may result in an energy allocation that differs from that seen
98 in mussels from the lower littoral zone (Petes et al., 2008; Tagliarolo et al., 2012).
99



100

101 **Figure 1.** Sampling sites for the seasonal (Rakkfjorden) and spatial (Simaviknes, Rakkfjorden,
102 Tromvik, Brensholmen, Oldervik and Buktalandet) studies.

The aim of the spatial study was to investigate the universality of the results from the seasonal study by examining the same biomarker responses in additional mussel populations from the lower littoral zone located within a 60-km radius from the site at Rakkfjorden (Fig. 1). We hypothesized that there would be no differences in biomarkers among populations because the mussels were collected within a short time period (4 days), they were expected to be at the same stage of reproductive development, the chosen sites were generally similar to each other in terms of physical characteristics (rocky shore and sandy bottom), and environmental conditions as they were supposed to represent relatively pristine environments with little background pollution.

The study is unique in its design as it completes the study from the same location performed by Nahrgang et al. (2013) with continuous logging of environmental conditions over the entire year, description of the reproductive cycle of the local mussels, their monthly levels of the baseline biomarkers, and the validation of the findings of this location across a spatial sampling. In addition, measurement of the baseline biomarker levels in the mussels from upper and lower littoral zone underlines the importance of the tidal zone for future biomonitoring studies previously reported only in few papers (Izagirre et al., 2008; Letendre et al., 2009; Schmidt et al., 2012).

2. Materials and methods

2.1 Environmental conditions

Environmental conditions at the mussel sampling site in Rakkfjorden were measured from 16th February 2013 to 18th April 2014 using air and underwater HOBO Pendant® Temperature/Light 64K Data loggers UA-002-64 deployed above the mussel bed and in the lower littoral zone. Light intensity, air temperature and water temperature were recorded every 5 min and data were then used to calculate weekly means (n=2016 data points per week). Salinity was recorded every 10 min in the lower littoral zone using a HOBO Conductivity/Salinity Data Logger U24-002-C. Water temperature and salinity were measured once at each site in the spatial study using the YSI Professional 1020 instrument (Pro1020).

132 Tidal information was obtained from the Norwegian Mapping Authority
133 (http://kartverket.no/en/sehavniva/) to determine the amount of time mussels were air exposed
134 during one tidal cycle (6 hours) in the upper and lower littoral zone.
135
136 2.2 Determination of total chlorophyll A concentration in seawater
137 For chlorophyll A (Chl A) quantification, in both seasonal and spatial studies (Table S1),
138 one litre of water was sampled from 20 cm depth in a green glass bottle, and then held cool and
139 dark for a few hours before being filtered. Three subsamples (300 ml) were vacuum-filtered on
140 Whatman GF/F filters (>0.7 µm), and Chl A was subsequently extracted in 5 ml methanol
141 overnight at 4 °C (Holm-Hansen and Riemann, 1978). The next day the concentration of Chl A
142 (mg/m^3) was measured using a Turner Design AU-10 fluorometer calibrated with pure Chl A
143 (Sigma, C6144).
144
145 2.3 Mussel collection
146 *Mytilus edulis* is the dominant mussel species in Rakkfjorden, but *M. galloprovincialis*
147 and some hybrids are also present (Mathiesen et al., 2016). It is difficult to distinguish between
148 the two species and their hybrids by visual inspection of their external shells, so the designation
149 *Mytilus* spp. will be used in this paper.
150 For the seasonal study, mussels (*Mytilus* spp.) were collected at 50 cm (upper littoral
151 zone) and 80 cm (lower littoral zone) below mean sea level, being out of water for 3.0±0.1 and
152 1.3±0.1 hours per tidal cycle, respectively, each month from 9th April 2013 to 16th April 2014
153 (Table S1). At each sampling point the largest mussels were selected (n=9–12 per month from
154 each tidal zone). Mussels were collected by hand during low tide and transported directly to the
155 laboratory for dissection. Mussels from the lower littoral zone were transported in a cool box
156 containing seawater, while the mussels from the upper littoral zone were transported in a cool
157 box that contained paper towels soaked in seawater to provide a cool and moist environment.
158 All mussels were used for histology and biomarker analyses (Table S1).
159 For the spatial study, mussels (*Mytilus* spp.; n=30 per site, except n=39 in Rakkfjorden)
160 were collected from the lower littoral zone of six sites (Fig. 1; Table S1) from 25th July to 28th
161 July 2014 with 12 or 24 hours between collection. The mussels were collected by hand during
162 low tide and transported in a cool box containing seawater to the laboratory for dissection.

163 Fifteen mussels from each site were used for histology and biomarker analyses (Table S1), and
164 three pools of 5–8 mussels from each site were prepared to determine the concentrations of
165 polycyclic aromatic hydrocarbons (PAHs) and metals in their soft tissues (Table S1) to evaluate
166 the degree of pollution.
167
168 ### 2.4 Dissection
169 In mussels collected for the seasonal study (n=9–12 per month from each tidal zone),
170 approximately 0.1 ml of haemolymph was removed from the posterior adductor muscle with a
171 syringe containing approximately 0.1 ml physiological saline (pH 7.3). Collected haemocytes
172 were used to measure LMS, a cellular biomarker for general organism health that assesses the
173 changes in lysosome membrane integrity as a response to contamination, using the neutral red
174 retention assay (Martínez-Gómez et al., 2015). The haemolymph/saline solution was gently
175 mixed and placed in a microcentrifuge tube, from which a 40 µl sample was pipetted onto the
176 centre of a microscope slide. The slide was left in a dark chamber for 15 min to allow adhesion
177 of the cells to the slide. The chamber contained crushed ice and paper towels soaked in water
178 on top of it to provide a cool and humid atmosphere. The average air temperature inside the
179 chamber stayed at 17.7±0.3 °C throughout the whole assay, as it was recommended in
180 Martínez-Gómez et al. (2015), and was likely stable over the year. Following incubation,
181 excess liquid was removed and 40 µl of neutral red solution was added. The neutral red
182 solution was taken up by the haemocyte lysosomes. The ability of the lysosome to retain the
183 neutral red solution was examined by inspection every 15 min for 1 hour and then every 30 min
184 for 2 hours at 40× magnification using a Leica DM2000 LED light microscope. The test was
185 terminated and the time recorded when over 50 % of the haemocyte lysosomes had leaked the
186 neutral red dye into the cytosol.
187 Shell length, width and height of all mussels (±0.01 mm) were measured using a digital
188 caliper. Length was defined as maximum anterior-posterior distance, width was measured from
189 top hinge joint to lower shell edge distance, and height was measured dorsoventrally at the
190 thickest part of the mussel (Fig. S1). Shell measurements were used to calculate the volume (V)
191 of the mussels as a simplified proxy of the size:
192 $V = 0.455 \times \text{length} \times \text{width} \times \text{height}$ (Versteegh, 2012).

193 Total wet weight (total soft tissues + shell) and shell weight (±0.0001 g) were recorded
194 using a Sartorius Extend ED124S analytical lab balance, and the total soft tissue wet weight
195 was computed as total wet weight minus shell weight. Condition index (CI) was then
196 calculated:

197 $$CI = 100 \times \frac{\text{soft tissue wet weight}}{\text{total wet weight}}$$ (Damiens et al., 2007).

198 Gonads were dissected out and fixed in 4 % formaldehyde for histological analysis.
199 Digestive glands were dissected out, weighed (±0.0001 g), snap frozen in liquid nitrogen and
200 stored at –80 °C until analysed for biomarkers (Table S1).
201

202 ## 2.5 Histological analysis

203 Gender and reproductive stage were determined using histological examination of gonads
204 (Table S1). Histological techniques were as described by Bancroft and Stevens (1982). Briefly, the
205 gonadal tissue was dehydrated in ethanol, cleaned in histoclear and then embedded in paraffin wax.
206 Thin sections (5 µm; n=5–10 per mussel) were prepared, stained with hematoxylin and eosin,
207 examined at 10× magnification by light microscopy, and photographed with a Pixera Pro 150ES
208 camera.
209 Reproductive stage was assigned to one of four stages (Maksimovich, 1985). Stage 0 was
210 the period of gonadal restoration after spawning, when the gonad was filled with connective
211 tissue and a few unreleased gametes (ova/spermatozoa) (Fig. 2A and 2B). Stage I was
212 designated early gametogenesis when follicular walls started to develop and immature gametes
213 (spermatogonia/oogonia) were formed (Fig. 2C and 2D). Stage II was considered to indicate
214 active gametogenesis when the follicules increased in size and contained developing gametes;
215 the follicules occupied about 50 % of the gonadal section (Fig. 2E and 2F). Finally, stage III
216 was designated as maturity, when follicules were of maximum size, filled with mature gametes
217 and inter follicular connective tissue was not visible (Fig. 2G and 2H).
218

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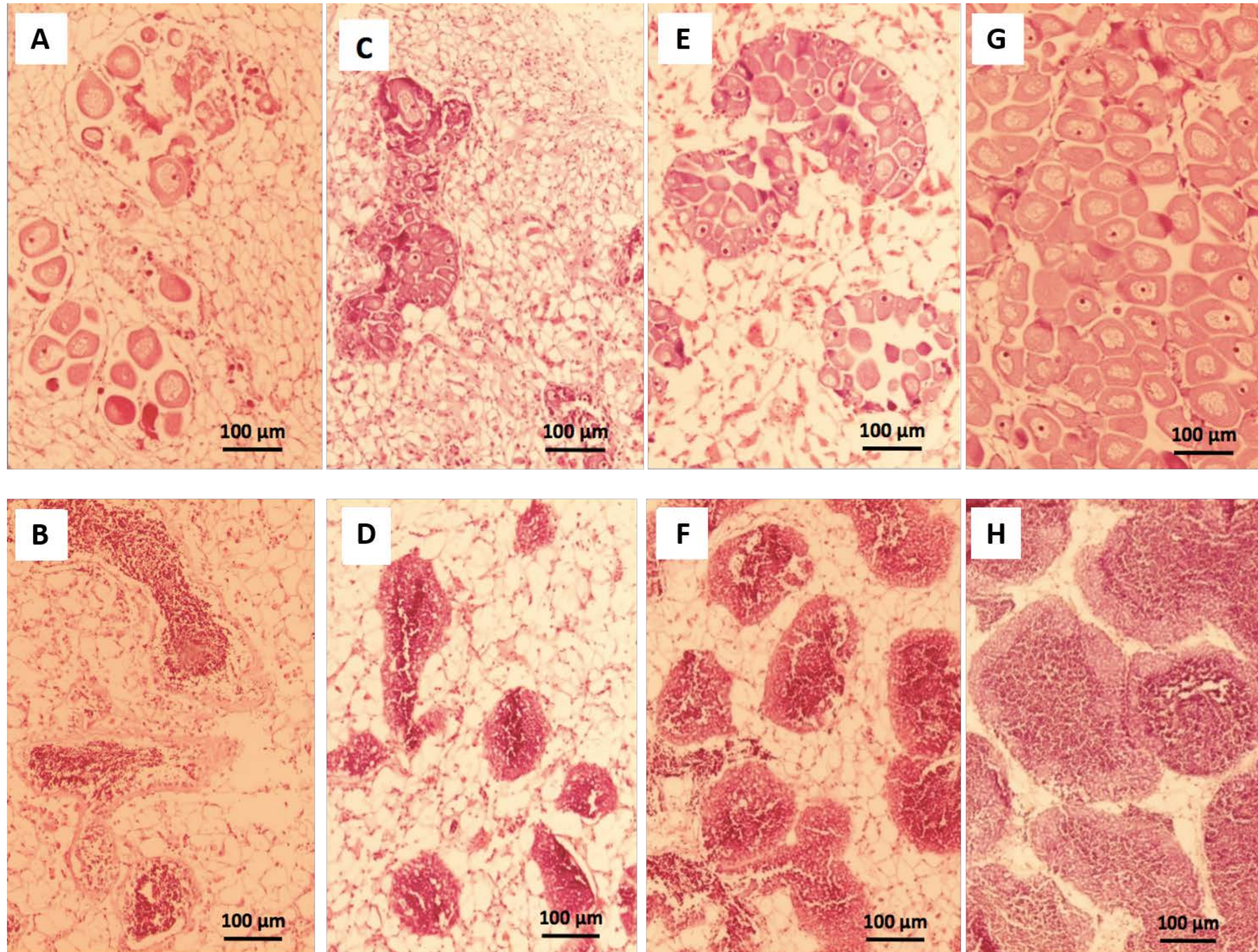


220 **Figure 2.** Developmental stages of *Mytilus* spp. female (A, C, E, G) and male (B, D, F, H) gonads collected for the seasonal study in
221 Rakkfjorden. (A and B) Stage 0 Gonadal restoration after spawning, July; (C and D) Stage I Early gametogenesis, November; (E and
222 F) Stage II Active gametogenesis, January; (G and H) Stage III Maturity and spawning, May. Magnification 10×.

223

224 2.6 Contaminant burden in soft tissues of the mussels collected for the spatial study

225 Determination of PAHs (26 PAHs in total) and metals in mussel soft tissues was
226 performed by Akvaplan-niva AS and the ALS laboratory group, respectively. Both laboratories
227 are accredited for the methods used.

228 The analysis of total PAHs included quantification of single analytes such as naphthalene,
229 phenanthrene/anthracene, dibenzothiophene, and their C1–C3 alkyl structural analogues (sum NPDs);
230 and sixteen 3–6 ring aromatics listed as priority pollutants by the Environmental Protection Agency
231 (16 EPA-PAHs). Two pools (5–8 mussels each) of mussel soft tissues from each site (only one pool
232 from Buktalandet) were used for the quantitative determination of total PAHs. Each pool (11–23 g
233 wwt) was weighed and a potassium hydroxide-methanol solution and an internal standard-mix of
234 deuterated PAHs were added. The solution was boiled under reflux for 4 hours (saponification),
235 filtered and extracted with pentane. The extract was dried with Na_2SO_4 (s), concentrated to 0.5 ml
236 and purified using gel permeation chromatography (GPC), with dichloromethane (DCM) as the
237 mobile phase, to remove fats and other impurities. After evaporation to approximately 0.5 ml and
238 solvent exchange to hexane, the extract was further purified on a solid phase extraction (SPE) column
239 by elution with pentane and DCM. Eluted solution was concentrated to 0.5 ml, evaporated to dryness
240 under N_2 gas, and finally 100 µl isooctane was added. Quantification of the final extract was carried
241 out using isotope-labeled internal standard calibration. Analyses were performed using Gas
242 Chromatography – Mass Spectrometry (GC-MS). Blind samples were run parallel to all samples, and
243 proficiency test samples (Quasimeme, Netherlands) were used as controls. The limit of detection
244 (LOD) was determined from analyses of a series of blank samples, processed along with real
245 samples, and calculated as: LOD = (blank average) + 3×(blank standard deviation). Half of the
246 detection limit value in compounds that were below LOD was used to calculate sums of NPDs and 16
247 EPA-PAHs.

248 Metal concentrations were analysed in the third pool (5–8 mussels) of mussel soft tissues
249 from each site. Frozen soft tissues (11–21 g wwt) were homogenized and then freeze-dried. The
250 dried samples were dissolved in concentrated nitric acid and hydrogen peroxide by microwave
251 digestion (170 °C, 30 min) in sealed Teflon vessels. Cooled samples were transferred to test
252 tubes and diluted to 10 ml. The concentration of As, Cd, Co, Cr, Cu, Hg, Mn, Ni, Pb and Zn
253 were measured by inductively coupled plasma sector field mass spectrometry (ICP-MS).

254 Analytical quality was confirmed through analyses of certified reference material bovine
255 muscle powder (NIST 8414).
256
257 2.7 Biochemical analyses of mussel digestive gland tissues
258 Digestive gland (100 mg) was homogenized in 1.6 ml potassium phosphate buffer (100
259 mM, pH 7.5) with 2.5 % NaCl using a precellys at 3 × 5000 rpm × 5 sec. For all biomarkers
260 except lipid peroxidation, the homogenates were subsequently centrifuged at 10000 *g* for 30
261 min at 4 °C. The obtained supernatants were split into subsamples for different biomarkers and
262 stored at −80 °C.
263 Lipid peroxidation, an indicator of oxidative stress on lipids in cells, was measured as
264 thiobarbituric reactive substances (TBARS) (Buege and Aust, 1978). The standard curve of
265 1,1,3,3-tetramethoxypropane (0–5 µM) and sample homogenates were mixed with TCA-TBA
266 reagent (trichloroacetic acid at 15 % w/v, thiobarbituric acid at 0.375 % w/v and HCl 37 %
267 diluted at 0.25N) in glass reagent tubes and incubated at 100 °C for 15 min. Tube content was
268 transferred to Eppendorf tubes and centrifuged at 1000 *g* for 10 min (4 °C). The supernatant
269 was pipetted in triplicates into a microplate. The absorbance was read at 532 and 600 nm with
270 the spectrophotometer plate reader. The absorbance at 532 nm was normalized to the
271 absorbance at 600 nm. The concentration was then calculated from the slope of the standard
272 curve and expressed as nmol TBARS/mg cytosolic protein.
273 CAT is an antioxidant enzyme that catalyzes the decomposition of hydrogen peroxide to
274 water and oxygen and protects cells from the oxidative stress. CAT activity was evaluated by
275 the method of Clairborne (1985), which is based on the disappearance of hydrogen peroxide
276 (H_2O_2) with time by the action of CAT contained in the examined samples. The decrease in
277 absorbance at 240 nm was recorded in a quartz cuvette every second for 1 min at 20 °C after
278 addition of 10 mM H_2O_2 to the twice-diluted sample homogenate and 50 mM phosphate buffer
279 (pH 7) using a spectrophotometer LAMBDA 35. CAT activity was measured in duplicate and
280 expressed in µmol/min/mg cytosolic protein.
281 GST is a pollution biomarker in bivalves (Brooks et al., 2011). This enzyme is generally
282 induced by oil-related compounds as it takes part in their detoxification. GST activity was
283 determined by measuring the increase in absorbance at 340 nm with time due to the
284 conjugation of glutathione (GSH) to 1-chloro-2,4-dinitrobenzene (CDNB) and the formation of

285 S-2,4-dinitrophenyl under the catalyzation of GST enzyme (Habig et al., 1974). The absorbance
286 was recorded in a quartz cuvette filled with twice-diluted sample homogenate, 20 mM CDNB,
287 20 mM GSH and 100 mM potassium phosphate buffer (pH 7.5) with 2.5 % NaCl. Recording
288 was made every second for 1 min at 20 °C. GST activity was measured in duplicate and
289 expressed as nmol/min/mg cytosolic protein.

290 The cytosolic protein concentration of the homogenates (mg/ml) was determined
291 according to Bradford (1976) using a bovine serum albumin standard curve (0–0.01 mg/ml).
292 Standard solution or sample homogenates diluted 1000× were pipetted in triplicates into
293 microwells. Coomassie blue reagent diluted 5× was added, and the plate was left for saturation
294 for 2 min. Absorbance was measured at 595 nm using the PerkinElmer Victor 3 plate reader.

295 The TOSC assay provides a measure of the biological resistance to oxidative stress
296 (Regoli and Winston, 1998). The TOSC was measured in digestive glands of the mussels from
297 lower littoral zone by quantifying the overall capability of cellular antioxidants to absorb
298 artificially generated oxyradicals, such as peroxyl (ROO·) and hydroxyl (OH). The
299 supernatants were ultracentrifuged at 50 000 *g* for 120 min at 4 °C and the cytosolic fraction
300 was stored at −80 °C until analysed. A separate reference was used for each sample, which was
301 measured with each run on the gas chromatograph. TOSC was measured in 55 µg of cytosolic
302 protein from each sample diluted in 800 µl of homogenizing buffer containing 2 mM α-keto-γ-
303 methiolbutyric acid and 200 mM 2,2'azobis (2-methylpropionamidine) dihydrochloride.
304 Glutathione was used as the positive control and homogenizing buffer was used as the blank.
305 Ethylene gas production was measured at intervals of 12 min from 36 to 84 min. The gas was
306 quantified by measurement with a GC-FID (6890N, Agilent Technologies, California, USA)
307 and a Supelco SPB-1 fused silica capillary column (30 m × 0.32 mm × 0.25 µm). Hydrogen, at
308 a flow rate of 1.5 ml/min, was the carrier gas with a split ratio of 1:20 with an inlet temperature
309 of 160 °C, oven temperature of 72 °C and a FID temperature of 220 °C. The TOSC values were
310 calculated using the equation: TOSC = (100–(∫SA/∫Blk × 100))/55, where "∫SA" is the integral
311 of the curve for the sample from the GC readout, "∫Blk" is the integral of the curve for the
312 blank from the GC readout. The TOSC values were normalised to cytosolic protein
313 concentration and expressed as Unit TOSC/mg cytosolic protein. The cytosolic protein
314 concentration of the samples was determined using the Lowry method (Lowry et al., 1951),

315 adapted for measurement with a plate reader and using bovine gamma globulin as the protein
316 standard.
317
318 2.8 Statistical analyses
319 Due to presence of multiple factors that potentially affected the seasonal variability of
320 biomarkers, the multivariate analyses Principal component analysis (PCA) and Projections to
321 Latent Structures (PLS) were performed on the data from the seasonal study using Simca P14.1
322 Software.
323 PCA was used for visualising the differences and similarities in the data set by
324 calculating principal components. These are mutually orthogonal vectors that represent
325 independent and uncorrelated variance of the initial descriptors (variables). Correlated
326 descriptors are described by the same principal component. The systematic variance in the data
327 set can hence be simplified by using fewer new descriptors than the original number of
328 variables, and this simplification is done without loss of systematic information (Carlson and
329 Carlson 2005). Since values of the descriptors varied in magnitude, the data were
330 logarithmically transformed and subsequently centred and scaled to unit variance in the
331 calculated PCA models. The number of significant components was determined by cross-
332 validation. R^2X is introduced as a measure of the fraction of the variation of X variables
333 explained by the model, and Q^2 is an estimate of the reliability/stability of the model calculated
334 by cross-validation. Score plots are obtained by projecting the original data onto the calculated
335 orthogonal principal component vectors. Variables with similar variance in their characteristics
336 are plotted close to one another. The influence of each original descriptor to the principal
337 component is reflected in a loadings plot. Descriptors which have a strong contribution to the
338 variation depicted in the score plot are found far from the origin in the loadings plot. Positively
339 correlated descriptors are projected close to each other, while negatively are projected opposite
340 to each other with respect to the axis centre.
341 The PLS models were run for all months, both tidal zones and both genders.
342 Reproductive stage 0 and I were combined in early maturity phase, while stage II and III were
343 combined in late maturity phase in order to increase number of mussels (n) per reproductive
344 stage. Tidal zone, and individual parameters of the mussels (shell volume, CI, gender, maturity

345 phase) were applied as predictor variables. Biomarkers (TBARS, CAT, GST, TOSC) were
346 applied as responses.

347 The validity of the models was expressed by the amount of the variance in the response
348 matrices ($R^2_{responses}$), which accordingly should be approaching 1. Calculated models with
349 $R^2_{responses}$ values below 0.4 were disregarded. Q^2 is the predictive power, an estimate of the
350 reliability/stability of the model calculated by cross-validation. In order to obtain a high
351 predictive power, $R^2_{responses}$ should be high. A value of $Q^2>0.9$ is excellent, while a value above
352 0.5 is good. With non-significant components, Q^2 can even be negative. The difference
353 between $R^2_{responses}$ and Q^2 should be as low as possible. The models with a difference in
354 $R^2_{responses}$ and Q^2 larger than 0.3 were considered unstable, as it may indicate outliers or the
355 presence of irrelevant predictor variables.

356 The output of the model included the variable importance in the projection (VIP) plots
357 presenting the importance of each variable in the model with respect to its correlation to all the
358 responses and to the projection. VIP plots reflected the relative importance of the model
359 variables to each other. Variables with VIP values greater than 1 were considered as variables
360 with high influence on the model, they were relevant for explaining the responses; variables
361 with VIP values in the range 0.5–1 were considered as variables with moderate influence on the
362 model; variables with VIP values less than 0.5 were considered as variables with low influence
363 on the model.

364 Microsoft Excel and software package XLSTAT Version 2016.03.30887 were used for
365 statistical analyses among group means. Assumptions of normality and homogeneity of all variances
366 were checked using normal P-plot and Levene's test, respectively. As neither assumption was
367 violated, one-way ANOVA was performed. Statistical significance was considered when $p\leq0.05$.

368 For the seasonal study, significant differences ($p\leq0.05$) in individual parameters of the mussels
369 (length, width, height, shell volume, CI) were tested across months (11), and for upper and lower
370 littoral zones. Significant differences ($p\leq0.05$) in biomarkers (TBARS, CAT, GST, TOSC) were
371 tested across months (11), for upper and lower littoral zones, for females and males, and for early and
372 late maturity phase. Total Chl A concentrations in water samples were tested for significance across
373 months (7).

374 For the spatial study, significant differences ($p\leq0.05$) in individual parameters of the mussels
375 (length, width, height, shell volume, CI) and biomarkers (TBARS, CAT, GST) were tested across

376 sites (6), for females and males, and across reproductive stages (4). Bioaccumulated concentrations
377 of 16 EPA-PAHs and NPDs in mussel soft tissues, as well as total Chl A concentrations in water
378 samples were tested for significance across sites (6). Statistical analyses were not run for metals as
379 the samples included only one replicate per sampling site.

380 Tukey´s post-hoc test was performed to make multiple pair wise comparisons and find which
381 pairs of months, sites and reproductive stages were significantly different ($p \leq 0.05$).

382 A chi-square test was used to compare observed sex ratios (number of females : number of
383 males) in the mussels from each of the tidal zones and from each of the site of the spatial study to the
384 theoretical proportion 1:1 (significance level $p \leq 0.05$).

385 Possible differences ($p \leq 0.05$) in observed distributions of reproductive stages (number of
386 mussels per reproductive stage) and LMS (number of mussels per neutral red retention time class)
387 were tested using a Fisher's exact test. For the seasonal study, the differences were tested across
388 months, for upper and lower littoral zones, for females and males from the same tidal zone and across
389 reproductive stages (only LMS). For the spatial study, the differences were tested across sites and for
390 females and males at each site.

391

392 3. Results

393 3.1 Seasonal study

394 3.1.1 Environmental conditions in Rakkfjorden

395 With its location at the 69th parallel north of the Arctic Circle, both photoperiod and light
396 intensity in Rakkfjorden vary significantly over the year. The highest light intensities (>10000
397 lux) were recorded from the end of May to the end of July, when the Sun is continuously above
398 the horizon (Fig. S2A). The lowest light intensities (<500 lux) were recorded from the
399 beginning of December to the beginning of February when the Sun is continuously below the
400 horizon.

401 The warmest period in Rakkfjorden in 2013 was recorded from the middle of May to the
402 middle of September, with weekly mean air temperatures ranging from 10 °C to 15 °C (Fig.
403 S2A). The lowest weekly mean air temperatures were recorded in December (–4 °C), January
404 (–11 °C) and March (both years of logging –6°C).

405 The weekly mean water temperature increased from 2.0±0.1 °C in March to 11.7±0.3 °C
406 in June, July and August, and the maximum water temperature (13.5±0.1 °C) was recorded at

407 the end of July (Fig. S2B). There was a gradual decrease in water temperature from 10.8±0.4
408 °C in September to 0.6±0.4 °C at the end of January.
409 Salinity changed both over time and with the tidal cycle due to the location of the mussel
410 bed between the fjord with seawater and a lagoon with freshwater, from snow melt and land
411 runoff. In February and March, salinity was relatively stable at 30.2±0.1 psu because the lagoon
412 was frozen, but in July, August and September, salinity fluctuated between 24.1±0.5 psu during
413 rising tides and 15.8±0.6 psu during falling tides (Fig. S3).
414
415 3.1.2 Total Chl A concentration in seawater
416 Significantly high levels of Chl A in seawater in Rakkfjorden were recorded in March
417 and April (0.9–1.4 mg/m^3; Fig. S4). The levels of primary production (0.5–0.7 mg/m^3)
418 remained elevated in May, June and September. In January, the total Chl A concentration was
419 the lowest (0.069±0.003 mg/m^3).
420
421 3.1.3 Description of collected mussels
422 Mussels in this study were selected by hand to be within the largest size range of their
423 specific tidal zone. Thus, mussels from the lower littoral zone were significantly larger than
424 mussels from the upper littoral zone in all months, except August, November and December
425 (Table 1; Table S2). Furthermore, mussels from the lower littoral zone had significantly higher
426 CI than mussels from the upper littoral zone in the winter and early spring month (April 2013,
427 December, January, March and April 2014) (Table 1). However, no major significant
428 differences in shell volume and CI were noted in any mussels across months (Table 1).
429

430 **Table 1.** Mean (±SE) volume and condition index (CI) of the mussels *Mytilus* spp. from upper and
431 lower littoral zones (n=9–12 per month from each tidal zone) collected for the seasonal study in
432 Rakkfjorden. Asterisk (*) for the mussels from lower littoral zone indicate significant differences
433 (ANOVA, p≤0.05) with the mussels from upper littoral zone. Letters (a, b, c) indicate significant
434 differences (ANOVA, p≤0.05) across months. No significant differences (ANOVA, p≤0.05) in CI
435 were found for mussels from upper littoral zone across months.

Month	Upper littoral zone		Lower littoral zone	
	Volume, ml	CI, %	Volume, ml	CI, %
April 2013	$4.9\pm0.3^{a,b}$	33.8±1.7	$6.0\pm0.2^{*,b}$	$39.1\pm1.8^{*,a,b}$
May	4.1 ± 0.3^{b}	37.8±2.4	$7.2\pm1.0^{*,a,b}$	37.2 ± 2.9^{b}
June	$4.7\pm0.4^{a,b}$	37.5±2.4	$7.4\pm0.7^{*,a,b}$	$42.0\pm1.8^{a,b}$
July	3.8 ± 0.3^{b}	37.6±1.3	$6.7\pm0.7^{*,a,b}$	$42.4\pm3.5^{a,b}$
August	$4.9\pm0.4^{a,b}$	35.8±1.5	$3.0\pm0.4^{*,c}$	$39.5\pm1.8^{a,b}$
September	$4.4\pm0.1^{a,b}$	38.1±1.2	$7.0\pm0.6^{*,a,b}$	$39.4\pm1.3^{a,b}$
November	$4.8\pm0.3^{a,b}$	36.7±1.2	$5.7\pm0.4^{b,c}$	36.6 ± 1.2^{b}
December	5.9 ± 0.5^{a}	37.1±2.7	$5.0\pm0.4^{b,c}$	$46.8\pm1.1^{*,a}$
January	$4.8\pm0.2^{a,b}$	32.9±1.2	$9.6\pm1.1^{*,a}$	$42.9\pm2.0^{*,a,b}$
March	4.1 ± 0.4^{b}	31.0±1.0	$7.3\pm0.4^{*,a,b}$	$41.6\pm1.1^{*,a,b}$
April 2014	3.7 ± 0.4^{b}	36.1±1.5	$7.7\pm0.8^{*,a,b}$	$43.0\pm0.7^{*,a,b}$

436

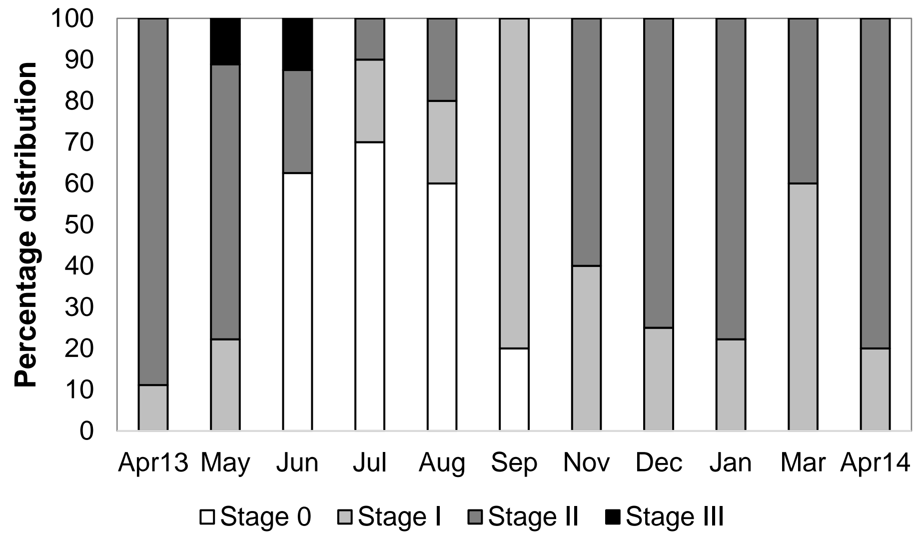
437 The sex ratios of the mussels from the upper littoral zone and from the lower littoral zone were
438 balanced (Table S1) with no significant differences between months (data not shown). Gender related
439 differences were only found sporadically for shell volume and CI in July (data not shown). The
440 distributions of the reproductive stages in the population of collected mussels (females and males
441 together) was similar (p>0.05) between tidal zones for all months, except in July, where a higher
442 proportion (70%) of stage 0 individuals were collected from the upper littoral zone, compared to the
443 lower littoral zone (13 %) (Fig. 3). The appearance of male mussels with mature gonads (stage III)
444 was recorded in January and April 2014 for the lower littoral zone, whereas in the upper littoral zone
445 it was firstly recorded in May.

446

A

	May	Jun	Jul	Aug	Sep	Nov	Dec	Jan	Mar	Apr14
Apr13	ns	*	**	*	***	ns	ns	ns	*	ns
May		*	**	ns	**	ns	ns	ns	ns	ns
Jun			ns	ns	**	**	*	*	**	**
Jul				ns	*	**	**	**	**	**
Aug					ns	*	*	*	*	*
Sep						**	**	**	*	**
Nov							ns	ns	ns	ns
Dec								ns	ns	ns
Jan									ns	ns
Mar										ns

447

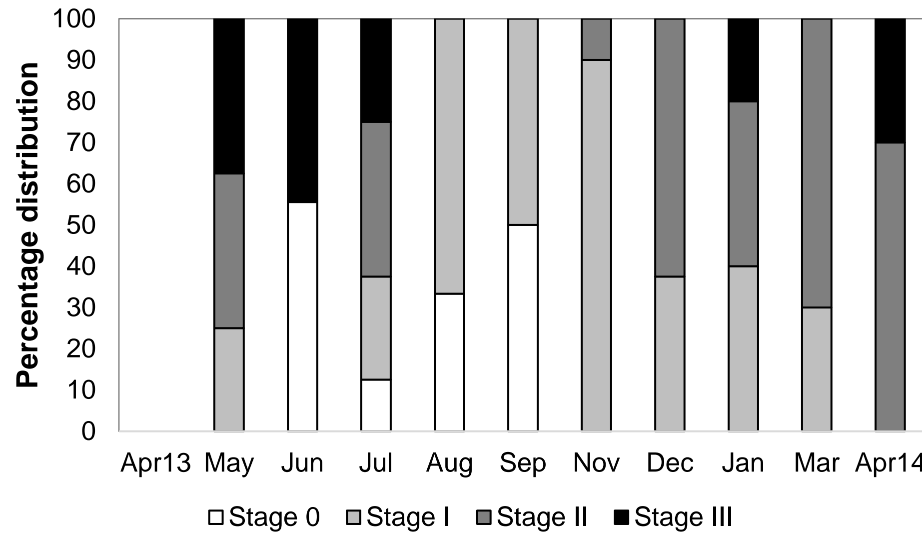


448

B

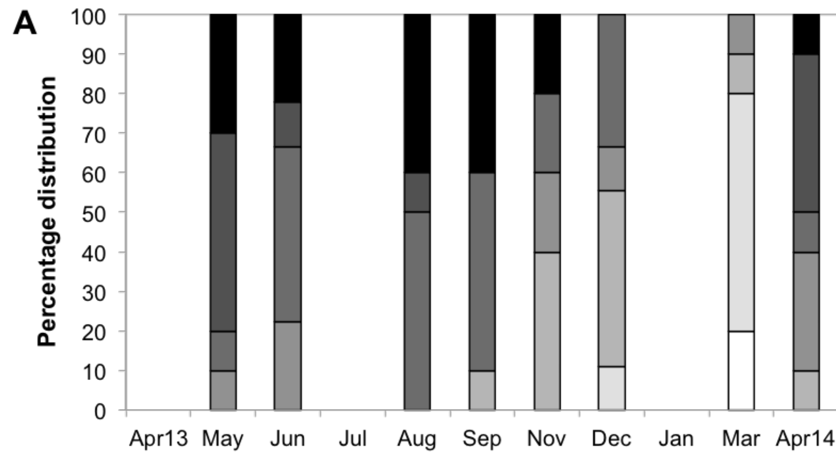
	Jun	Jul	Aug	Sep	Nov	Dec	Jan	Mar	Apr14
May	*	ns	ns	**	*	ns	ns	ns	ns
Jun		*	*	*	***	***	**	***	**
Jul			ns	*	*	ns	***	ns	ns
Aug				ns	ns	ns	ns	*	**
Sep					**	**	**	***	***
Nov						*	ns	**	***
Dec							ns	ns	*
Jan								ns	ns
Mar									*

449

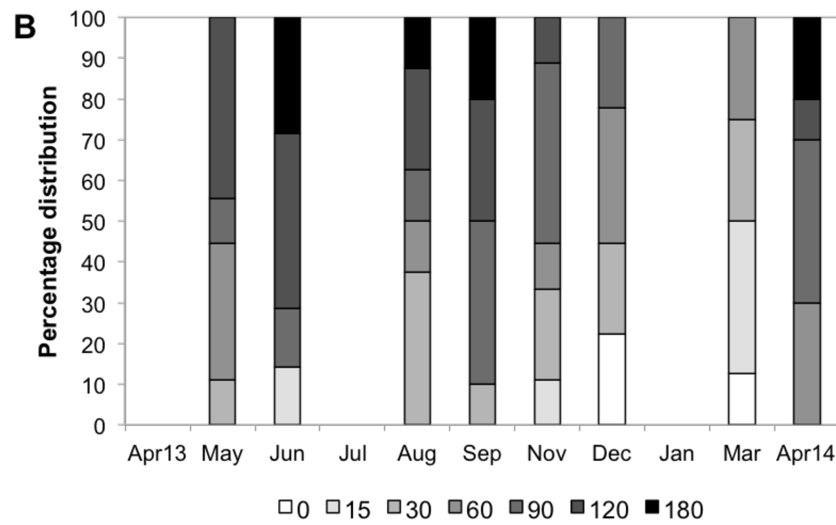


450

451 **Figure 3.** Distribution of reproductive stages of mussels *Mytilus* spp. from upper (A) and lower (B)
452 littoral zones (n=9–12 per month from each tidal zone) collected for the seasonal study in
453 Rakkfjorden. The inserted table indicates significant differences (Fisher's exact test; ns = non-
454 significant, *p<0.05, **p<0.01, ***p<0.001) across months.
455
456 The mussels had mature gonads (stage III) between April (lower littoral zone)/May
457 (upper littoral zone) and July and spawned between June and September (Fig. 3). In June and
458 September, gonads were spent (stage 0). Gonads in early gametogenesis (stage I) were
459 observed in all months, except June, and gonads in active gametogenesis (stage II) were present
460 in all months, except September.
461
462 3.1.4 Biomarker responses
463 There was a significant seasonal variation in LMS in mussel haemocytes from both tidal
464 zones, with higher neutral red retention time (90–180 min) in the summer half-year (April to
465 September) than in the winter half-year (November, December and March, 0–60 min) (Fig. 4).
466 No differences in LMS were found for mussels from the upper and lower littoral zones in any
467 month, neither across reproductive stages in any month.
468



	May	Jun	Jul	Aug	Sep	Nov	Dec	Jan	Mar	Apr14
Apr13										
May		ns		ns	*	*	**		***	ns
Jun				ns	ns	ns	ns		***	ns
Jul										
Aug					ns	*	*		***	*
Sep						ns	ns		***	*
Nov							ns		**	ns
Dec									*	ns
Jan										
Mar										***



	May	Jun	Jul	Aug	Sep	Nov	Dec	Jan	Mar	Apr14
Apr13										
May		ns		ns	ns	ns	ns		ns	ns
Jun				ns	ns	ns	*		*	ns
Jul										
Aug					ns	ns	ns		ns	ns
Sep						ns	*		**	ns
Nov							ns		ns	ns
Dec									ns	ns
Jan										
Mar										*

469 **Figure 4.** Distribution of lysosome membrane stability (LMS) measured as neutral red retention time (min) in haemocytes of
470 mussels *Mytilus spp.* from upper (A) and lower (B) littoral zones (n=9–12 per month from each tidal zone) collected for the
471 seasonal study in Rakkfjorden. The inserted tables indicate significant differences (Fisher's exact test; ns = non-significant,
472 *p<0.05, **p<0.01, ***p<0.001) across months.

473

474 Only a few differences in biomarkers, such as TBARS, CAT, GST and TOSC, were
475 found among months, between tidal zones, between genders, and between early and late
476 maturity phase. As part of an initial assessment, PCA models were used to visualize potential
477 differences and similarities between exogenous variables (month, light intensity, water
478 temperature, salinity, Chl A, tidal zone), endogenous variables (shell volume, CI, gender,
479 reproductive stage/maturity phase) and the biomarker responses. Including all variables and
480 responses resulted in poor PCA models, describing less than 40 % of the variance in the
481 dataset. For this reason, variables and responses were analysed separately. The first PCA model
482 included all the exogenous and endogenous variables and the X-axis explained 53 % of their
483 variance (Fig. 5A) with clusters according to the months (Class 1-11). The loadings plot
484 revealed that exogenous factors (light intensity, water temperature, salinity and Chl A) had a
485 strong influence on the variance in X-axis (Fig. 5B). Since these exogenous factors were equal
486 on the same month, these variables might result in skewed representation of the variance in the
487 PCA model. Based on this, a new PCA model was calculated, which only included variables
488 that varied on and between the months (CI, shell volume, tidal zone, reproductive
489 stage/maturity phase and gender). The model explained 56 % of the variance and no clustering
490 according to the month was observed (Fig. 5C). The loadings plot showed that the tidal zone,
491 CI and shell volume had the highest influence on the variance in X-axis (explaining 29 % of the
492 variance) and reproductive stage/maturity phase had the highest influence on the variance in Y-
493 axis (explaining 27 % of the variance) (Fig. 5D).The PCA model of the biomarker responses
494 explained 54 % of the variance and there was clustering according to the month (Fig. 5E). This
495 indicated that the trend in biomarker responses varied according to the biomarker. TBARS and
496 CAT had the highest influence on the variance in X-axis, while TOSC-ROO· had the highest
497 influence on the variance in Y-axis (Fig. 5F).
498

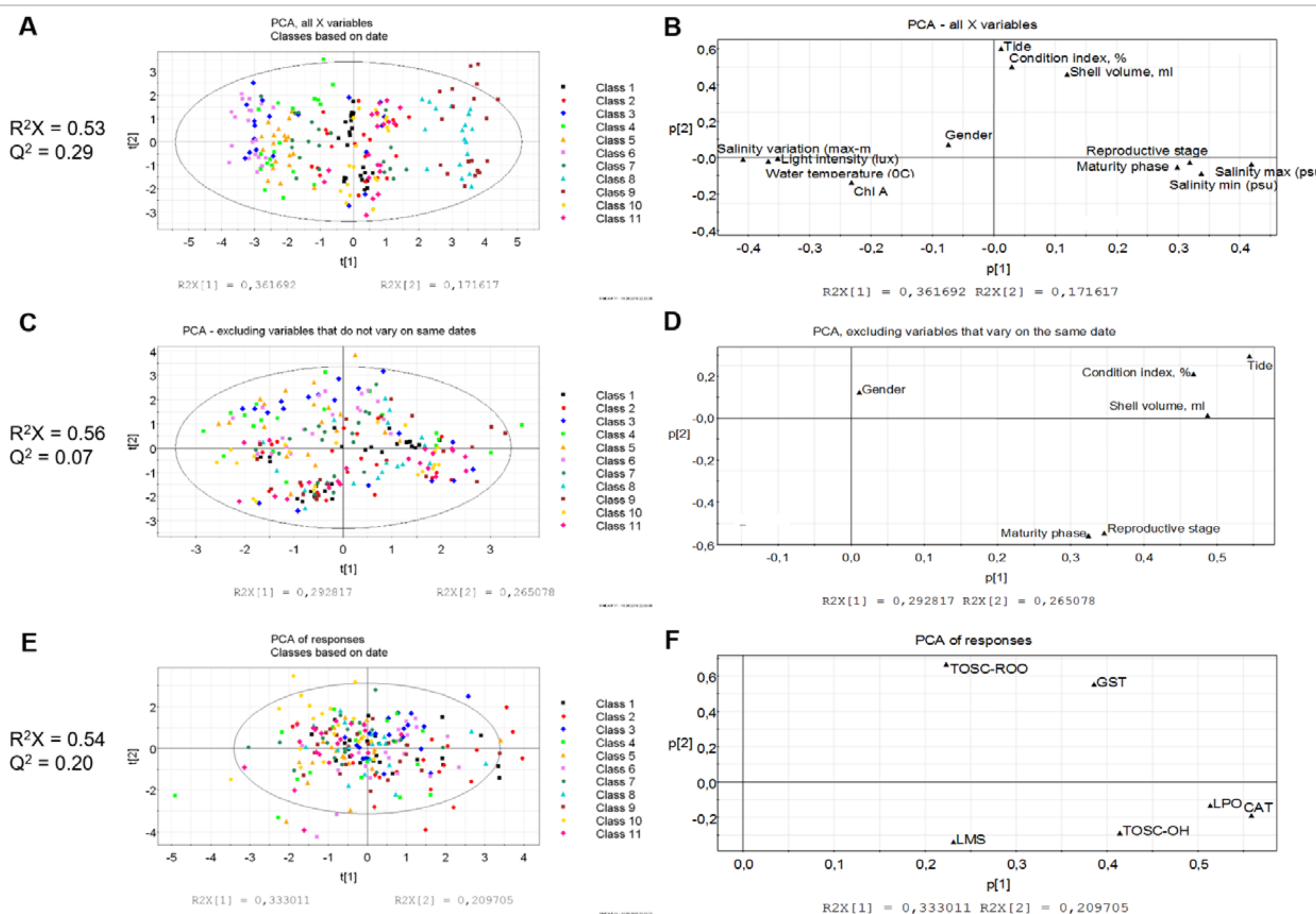


Figure 5. Results of the principal component analysis (PCA) models: scores plots (A, C, E) and loadings plots (B, D, F). R^2X indicates a measure of the fraction of the variation of X variables explained by the model. Q^2 is an estimate of the reliability/stability of the model calculated by cross-validation.

503

504 The PLS models were plotted to visualise effect of the tidal zone/shell volume/CI, gender
505 and maturity phase on each of the biomarkers.

506 In the model for TBARS, the gender was identified as an important factor for the
507 biomarker in June and December (Fig. 6). In June, males from the upper littoral zone had
508 significantly higher (1.2±0.1 nmol/mg protein) TBARS levels than females (0.69±0.04
509 nmol/mg protein). However, no significant differences in TBARS between genders were found
510 in December. High importance of the maturity phase as variable in the model was identified in
511 mussels in March (Fig. 6), when in the upper littoral zone males in the early maturity phase had
512 lower TBARS levels (stage I; 0.6±0.1 nmol/mg protein) than males in the late maturilty phase
513 (stage II; 1.2 nmol/mg protein). Also, the PLS indicated that the tidal zone and the CI would
514 affect the TBARS levels in December, even though the difference was weak and not significant
515 ($p>0.05$) based on the ANOVA (Fig 7A). The seasonal trend for TBARS levels was similar for
516 mussels from both tidal zones, with highest TBARS levels observed in May (3.4±0.7 nmol/mg
517 protein) (Fig. 7A). In all other months, the TBARS levels were not significantly different from
518 each other (mean of 0.91±0.04 nmol/mg protein).
519

TBARS

	Apr13	May	Jun	Jul	Aug	Sep	Nov	Dec	Jan	Mar	Apr14
$R^2_{responses}$			0.6					0.4		0.4	
Q^2			0.2					−0.1		−0.1	
Condition index			0.4					1.4		0.3	
Gender			1.9					1.3		0.7	
Maturity phase			0.6					0.8		1.0	
Shell volume			0.5					0.6		0.0	
Tidal zone			0.6					1.3		0.4	

CAT

	Apr13	May	Jun	Jul	Aug	Sep	Nov	Dec	Jan	Mar	Apr14
$R^2_{responses}$				0.5			0.6				
Q^2				0.5			0.4				
Condition index				0.1			0.6				
Gender				0.9			0.7				
Maturity phase				1.0			1.6				
Shell volume, ml				0.7			0.0				
Tidal zone				1.1			1.1				

GST

	Apr13	May	Jun	Jul	Aug	Sep	Nov	Dec	Jan	Mar	Apr14
$R^2_{responses}$	0.4								0.5		
Q^2	0.2								0.2		
Condition index	0.7								1.4		
Gender	0.1								0.9		
Maturity phase	0.7								0.1		
Shell volume	1.7								1.2		
Tidal zone	1.3								1.5		

TOSC-ROO

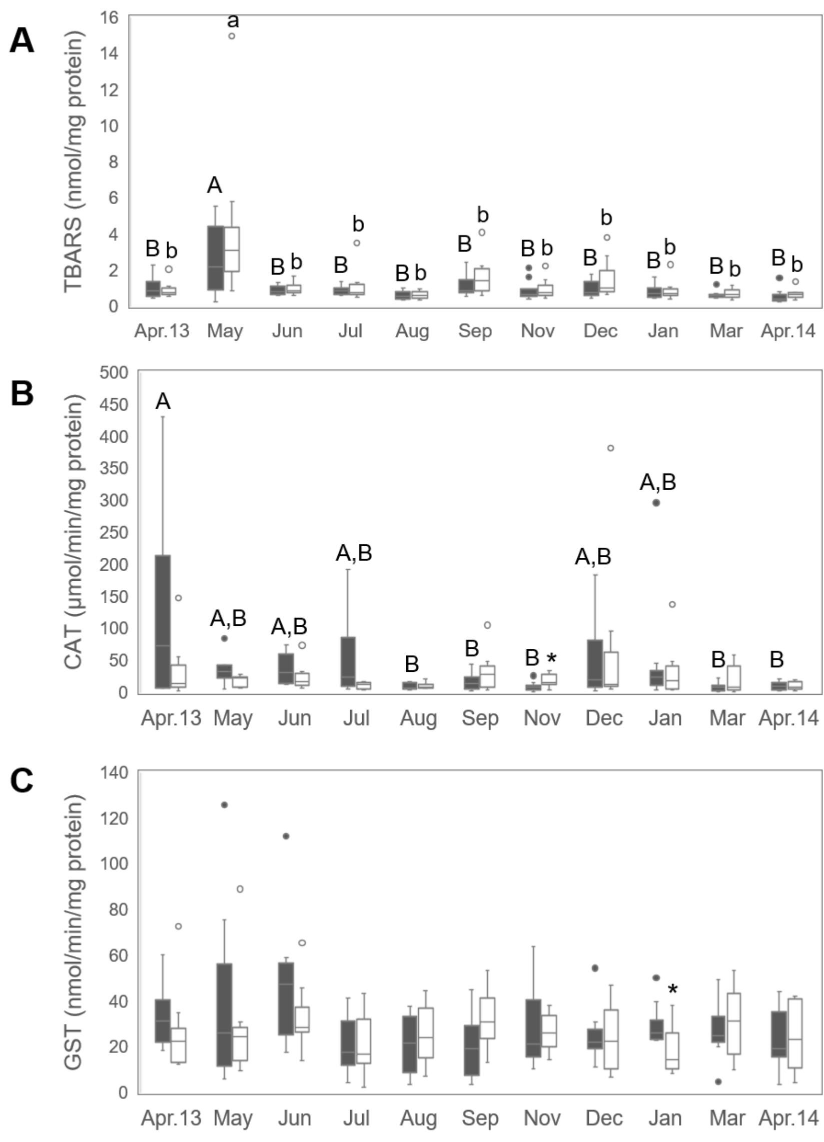
	Apr13	May	Jun	Jul	Aug	Sep	Nov	Dec	Jan	Mar	Apr14
$R^2_{responses}$	0.7		0.7			0.7	0.5		0.7	0.4	
Q^2	0.7		0.1			0.0	0.1		0.4	0.0	
Condition index	1.6		0.8			1.2	1.5		1.7	0.7	
Gender			2.1			0.4	0.6		1.2	1.7	
Maturity phase			0.6				1.4		1.3	0.4	
Shell volume	1.8		0.9			1.4	0.2		0.3	1.5	

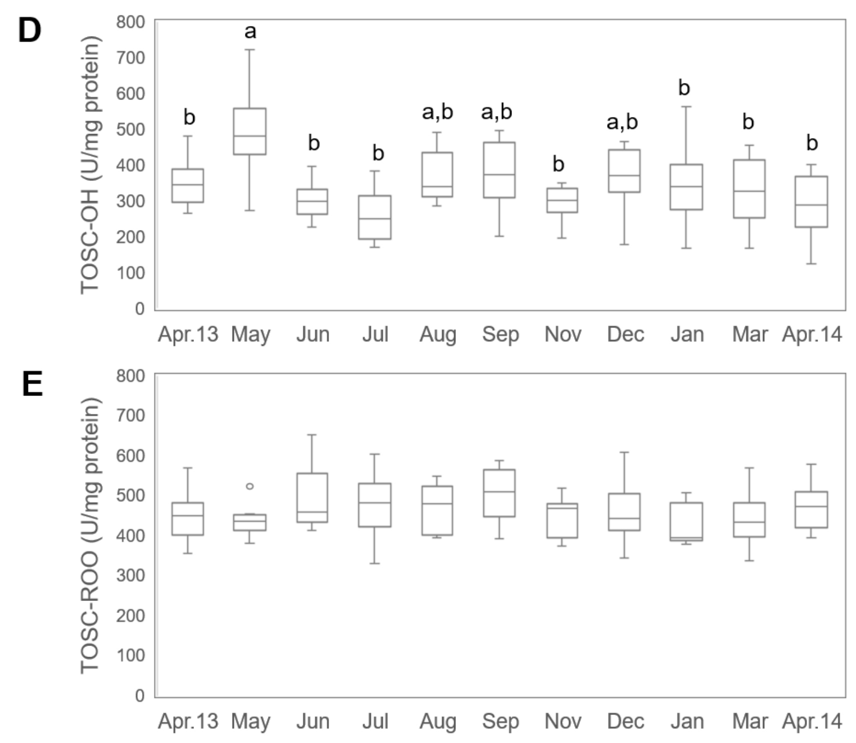
TOSC-OH

	Apr13	May	Jun	Jul	Aug	Sep	Nov	Dec	Jan	Mar	Apr14
$R^2_{responses}$		0.7	0.3	0.8				0.4			
Q^2		0.5	0.1	−0.1				−0.1			
Condition index		1.3	1.1	1.0				1.5			
Gender		1.2	0.1	1.3				0.9			
Maturity phase		1.2	0.6	1.3				1.1			
Shell volume		0.7	1.9	0.7				1.1			

Figure 6. Results of the projections to latent structures (PLS) models presented as variable importance in the projection (VIP) values. The models were run for all data of the seasonal study. Tidal zone and individual parameters of the mussels (condition index, gender, maturity phase and shell volume) were applied as predictor variables. Biomarkers (lipid peroxidation measured as

525 thiobarbituric reactive substances (TBARS), catalase (CAT) activity, glutathione S-transferase (GST)
526 activity, total oxyradical scavenging capacity towards ROO· radical (TOSC-ROO·) and OH radical
527 (TOSC-OH)) were applied as responses. Black cells indicate variables with high influence on the
528 model with VIP values greater than 1; grey cells indicate variables with moderate influence on the
529 model with VIP values in the range 0.5–1; light grey cells indicate variables with low influence on
530 the model with VIP values less than 0.5; white cells indicate poor models or no data. $R^2_{responses}$
531 indicates amount of variance explained in the response matrices, i.e. biomarkers (TBARS, CAT,
532 GST, TOSC-ROO·, TOSC-OH). Q^2 is an estimate of the reliability/stability of prediction of the PLS
533 model calculated by cross-validation.
534





535

536 **Figure 7.** Biomarkers measured in digestive gland of the mussels *Mytilus* spp. from upper littoral zone (grey) and lower littoral zone
537 (white) (n=9–12 per month from each tidal zone) collected for the seasonal study in Rakkfjorden. (A) Lipid peroxidation measured as
538 thiobarbituric reactive substances (TBARS); (B) Catalase (CAT) activity; (C) Glutathione S-transferase (GST) activity; Total
539 oxyradical scavenging capacity (TOSC) toward (D) peroxyl radical (TOSC-ROO·) and (E) hydroxyl radical (TOSC-OH). Plots
540 represent the median (line), 25%-75% percentiles (box), min-max range (whisker) and moderate outliers (dots). Asterisk (*) for
541 mussels from the lower littoral zone indicate significant differences (ANOVA, $p \leq 0.05$) with mussels from the upper littoral zone. No
542 significant differences (ANOVA, $p \leq 0.05$) in TBARS were found between mussels from the upper littoral zone and mussels from the
543 lower littoral zone. Letters (A, B for mussels from the upper littoral zone, and a, b for mussels from the lower littoral zone) indicate
544 significant differences (ANOVA, $p \leq 0.05$) across months. No significant differences (ANOVA, $p \leq 0.05$) in GST were found in mussels
545 from the upper littoral zone across months. No significant differences (ANOVA, $p \leq 0.05$) in CAT, GST and TOSC-ROO· were found
546 in mussels from the lower littoral zone across months.

547

548 In the model for CAT activity, maturity phase and tidal zone had a high influence on the
549 biomarker in July and November (Fig. 6). Significant differences in CAT activity in relation to
550 these two factors were found only in mussels collected in November. Indeed, in the upper
551 littoral zone, males in early maturity phase had higher (26.2 μmol/min/mg protein) CAT
552 activity than males in late maturity phase (5.3±1.6 μmol/min/mg protein). Also, higher CAT
553 activity was measured in the mussels from the lower littoral zone compared to the upper littoral
554 zone only in November (Fig. 7B). CAT activity was relatively stable across months. The only
555 remarkable change in CAT activity was significantly increased levels in mussels from the
556 upper littoral zone in April 2013 (120.7±43.7 μmol/min/mg protein) compared to August,
557 September, November, March and April 2014 (mean of 10.4±1.2 μmol/min/mg protein; Fig.
558 7B).

559 In the model for GST activity, high importance of the tidal zone/shell volume/CI was
560 recorded in April 2013 and January (Fig. 6). Indeed, the GST activity was higher in mussels
561 with smaller shell volume and lower CI (Table 1) from the upper littoral zone (upper 29.2±2.8
562 nmol/min/mg protein vs. lower 18.3±3.1 nmol/min/mg protein) (Fig. 7C). No significant
563 differences in the GST activity were observed in any tidal zone across months (mean of
564 27.4±1.2 nmol/min/mg protein).

565 In the models for TOSC, the importance of the variables was slightly different between
566 two radicals depending on the month (Fig. 6). However, it seemed that all individual
567 parameters of the mussels (CI, shell volume, gender and maturity phase) had high influence on
568 the biomarker. TOSC values for neutralisation of the ROO· radical (TOSC-ROO·) were not
569 significantly different across months (mean of 460.9±6.2 U/mg protein; Fig. 7D). TOSC values
570 for neutralisation of the OH radical (TOSC-OH) was higher in mussels collected in May
571 (489.0±37.4 U/mg protein) than in April 2013, June, July, November, January, March and
572 April 2014 (mean of 310.5±9.4 U/mg protein) (Fig. 7E).

573

574 3.2 Spatial study

575 3.2.1 Environmental conditions

576 Similar weather conditions (wind of 2–5 m/s, low-level clouds, fog and drizzle) and air
577 temperatures (12–16 °C) were recorded at all sites for all sampling days. Water temperature

578 was lower in Simaviknes (9.2 °C) and Oldervik (10.4 °C) than at the other sites (mean of
579 14.6±0.9 °C), possibly because the sampling was done during the morning hours (Table S3).
580 Salinity of 31–34 psu was measured in Simaviknes, Rakkfjorden, Tromvik and Brensholmen
581 (Table S3). Salinity was low in Oldervik (5 psu) and Buktalandet (7 psu) due to the arrival of
582 freshwater from the land runoff and river, respectively (Table S3).
583
584 3.2.2 Total Chl A concentration in seawater
585 The highest concentration of Chl A (8.2±0.1 mg/m^3) was measured in Simaviknes (Fig.
586 S5). At this site, a high concentration of green algae were observed in the water. In Tromvik,
587 Brensholmen and Buktalandet, the Chl A ranged between 2.2 and 2.9 mg/m^3. In Oldervik and
588 Rakkfjorden, the Chl A concentrations were the lowest (1.1–1.5 mg/m^3).
589
590 3.2.3 Description of collected mussels
591 The sex ratio was even among the mussels collected in Simaviknes (females n=7, males
592 n=8) and Oldervik (females n=8, males n=7). Females were dominant in samples collected in
593 Tromvik (females n=11, males n=4), whereas there were more males than females in
594 Rakkfjorden (females n=4, males n=11), Brensholmen (females n=5, males n=10) and
595 Buktalandet (females n=5, males n=10). No differences in shell volume and CI were found
596 between genders at any site.
597 Mussels collected in Oldervik, Tromvik and Brensholmen were significantly larger in
598 volume (min–max 11.0–28.3 ml) than mussels collected from the other three sites (min–max
599 7.9–18.3 ml; Table 2). The CI tended not to be different across the sites (Table 2).
600
601 **Table 2.** Mean (±SE) volume and condition index (CI) of mussels *Mytilus* spp. (n=15 from each site)
602 collected in six sites of the spatial study. Letters (a, b, c) indicate significant differences (ANOVA,
603 p≤0.05) across sites.

Site	Volume, ml	CI, %
Simaviknes	12.8±0.7[b]	47.5±1.1[a]
Rakkfjorden	11.0±0.7[b]	45.6±2.1[a,b]
Tromvik	18.8±1.2[a]	42.4±0.8[a,b,c]
Brensholmen	16.7±0.9[a]	41.9±1.2[b,c]
Oldervik	17.7±0.8[a]	38.7±1.2[c]
Buktalandet	11.4±0.5[b]	44.9±1.3[a,b]

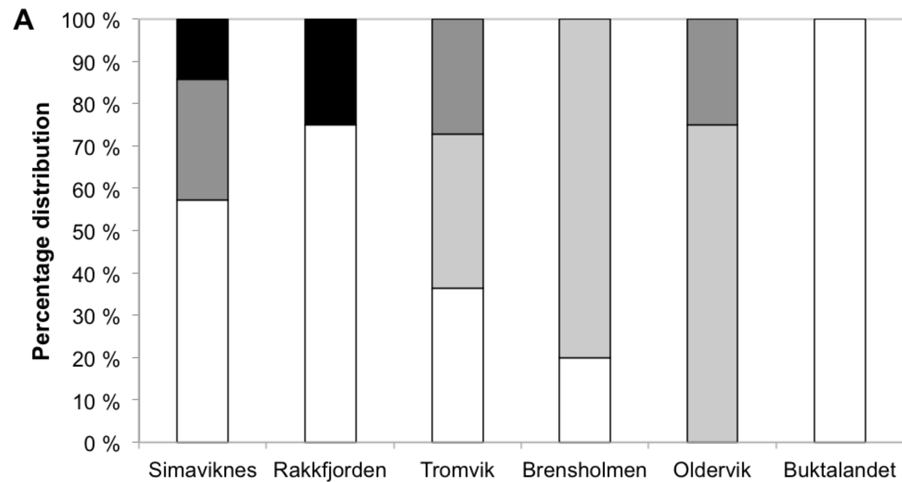
604

605 The significant dominance of individuals at stage 0 was observed for females (n=3) and males
606 (n=7) from Rakkfjorden, females from Simaviknes (n=4) and females from Buktalandet (n=5) (Fig.
607 8). Stage I was presented in most of the mussels from Brensholmen (females n=4, males n=9),
608 Oldervik (females n=6, males n=6) and in males from Buktalandet (n=9). Individuals at stage II were
609 dominated among males from Simaviknes (n=5). Distributions of the reproductive stages were
610 similar for both genders from Tromvik.

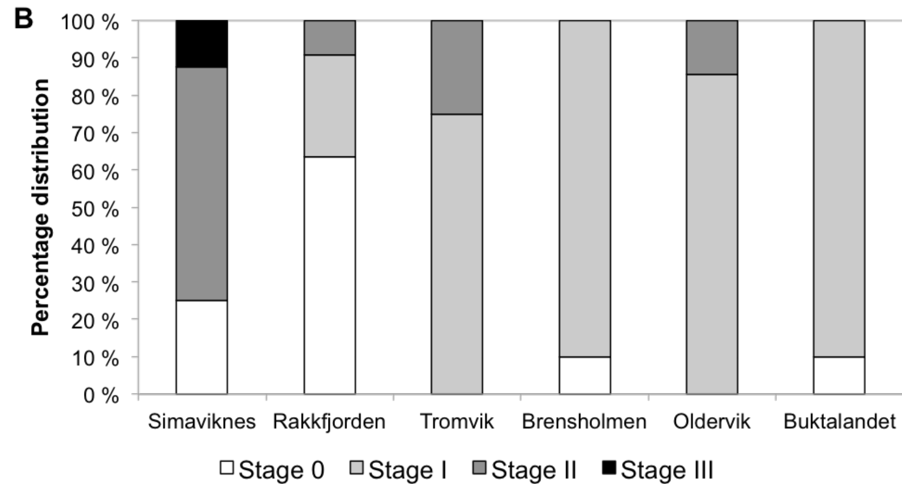
611 Distributions of reproductive stages of females and males were significantly different
612 only in Buktalandet (females (n=5) were at stage 0, whereas males were at stage 0 (n=1) and I
613 (n=9)) (Fig. 8).

614 No differences in shell volume or CI were found across reproductive stages at any site.

615



	Rakkfjorden	Tromvik	Brensholmen	Oldervik	Buktalandet
Simaviknes	ns	ns	*	**	ns
Rakkfjorden		ns	ns	**	ns
Tromvik			ns	ns	ns
Brensholmen				ns	*
Oldervik					***



	Rakkfjorden	Tromvik	Brensholmen	Oldervik	Buktalandet
Simaviknes	*	ns	***	**	***
Rakkfjorden		ns	*	*	*
Tromvik			ns	ns	ns
Brensholmen				ns	ns
Oldervik					ns

616 **Figure 8.** Distribution of reproductive stages of female (A) and male (B) mussels *Mytilus* spp. (n=15 per site) collected in six sites of
617 the spatial study. The inserted tables indicate significant differences (Fisher's exact test; ns = non-significant, *p<0.05, **p<0.01,
618 ***p<0.001) across sites.

619

620 ### 3.2.4 Contaminant burden in total soft tissues of the mussels

621 No significant differences in concentration of 16 EPA-PAHs in mussel soft tissues were
622 found across sites (Table 3). According to the Norwegian Environmental Agency classification
623 of environmental quality status based on the contaminant burden in blue mussels (TA-
624 1467/1997), the degree of pollution of the sum 16 EPA-PAHs in all sites was ranked as
625 moderate (Class II – No toxic effects). The levels of NPDs bioaccumulated in mussels were
626 similar at all sites, with the highest concentration in Brensholmen (Table 3). There is no quality
627 standard including background levels of NPDs.

628 The levels of metals in mussel soft tissues were relatively similar across all sites too. Zn
629 was the dominant metal followed by As, Cu and Mn (Table 3). The degrees of pollution of Cr,
630 Hg, Ni and Pb in Rakkfjorden, Simaviknes and Buktalandet were ranked as moderate (Class II
631 – No toxic effects), while As, Cd, Cu and Zn were ranked as marked (Class III – Chronic
632 effects) (TA-1467/1997). The site in Tromvik differed from Rakkfjorden only in reduced
633 concentration of Zn (Class II – No toxic effects). The mussels from Buktalandet had elevated
634 levels of Pb (Class III – Chronic effects) and reduced levels of Zn (Class II – No toxic effects)
635 compared to the mussels from Rakkfjorden. The site in Brensholmen was the most different in
636 metal levels compared to Rakkfjorden, showing lower levels of Cr (insignificant degree of
637 pollution, Class I – Background level), Cd, Cu and Zn (Class II – No toxic effects), but higher
638 levels of Pb (Class III – Chronic effects). There are no quality standards including background
639 levels of Co and Mn.

640

641 **Table 3.** Sum of 16 priority polycyclic aromatic hydrocarbons listed by the Environmental Protection Agency (16 EPA-PAHs), sum
642 of naphthalene, phenanthrene/anthracene, dibenzothiophene, and their C1–C3 alkyl structural analogues (NPDs) (both n=2 pools of 5–
643 8 mussels from each site, except n=1 from Buktalandet, mean±SE, µg/kg soft tissue wet weight) and metals (n=1 pool of 5–8 mussels
644 from each site, mean±SD, mg/kg soft tissue wet weight) in soft tissue pools from mussels *Mytilus* spp. collected in six sites of the
645 spatial study. Letters (a, b) indicate significant differences (ANOVA, p≤0.05) across sites. No significant differences (ANOVA,
646 p≤0.05) were found for 16 EPA-PAHs across sites. Statistical analyses were not run for metals.

	Simaviknes	Rakkfjorden	Tromvik	Brensholmen	Oldervik	Buktalandet
Sum 16 EPA-PAHs	102±26	82±16	54±2	99±5	97±38	79
Sum NPDs	235±21[b]	389±71[b]	231±37[b]	849±93[a]	470±78[a,b]	601[a,b]
As	13.4±3.6	19.1±5.1	16.8±4.5	14.2±3.8	9.4±2.5	11.2±3.0
Cd	1.5±0.3	1.2±0.2	1.8±0.3	0.9±0.2	1.2±0.2	1.3±0.2
Co	0.2±0.1	0.4±0.1	0.3±0.1	0.2±0.1	0.4±0.1	0.9±0.2
Cr	0.7±0.2	1.1±0.3	0.9±0.2	0.5±0.1	1.0±0.3	1.2±0.3
Cu	6.4±1.2	6.0±1.1	7.5±1.4	5.8±1.1	6.5±1.2	7.7±1.4
Hg	0.05±0.02	0.14±0.05	0.09±0.03	0.05±0.02	0.04±0.02	0.07±0.02
Mn	4.4±0.8	4.6±0.9	4.1±0.8	3.6±0.7	6.9±1.3	9.8±1.8
Ni	1.0±0.3	1.6±0.4	2.4±0.6	1.4±0.4	1.0±0.3	2.3±0.6
Pb	1.4±0.3	0.9±0.2	1.0±0.2	0.4±0.1	0.6±0.1	0.4±0.1
Zn	108±21	100±20	69±14	64±13	101±20	74±14

647

648

649 3.2.5 Biomarker responses

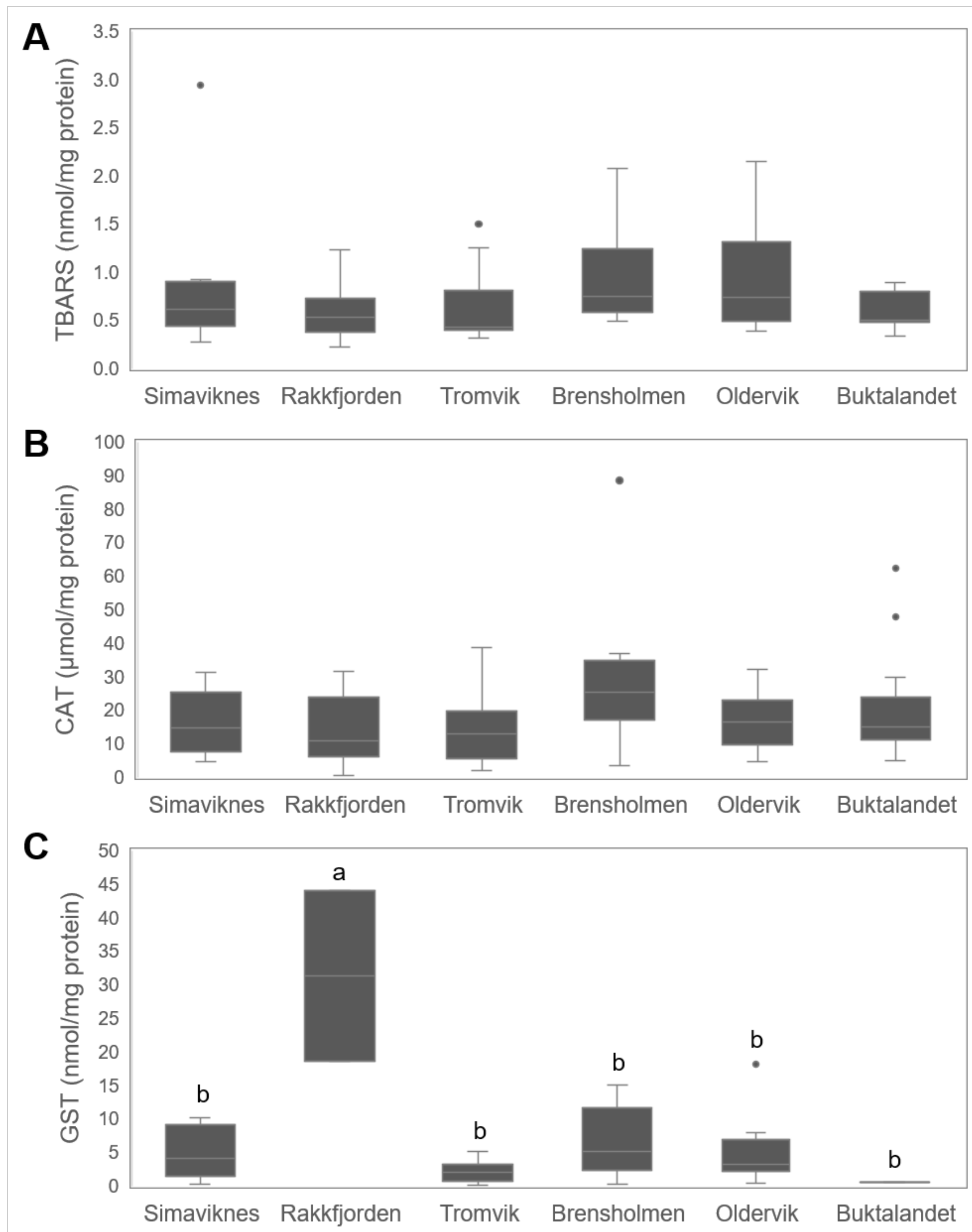
650 TBARS levels, CAT activity and GST activity measured in mussels from Rakkfjorden in
651 the spatial study were in the same range as in mussels collected for the seasonal study from the
652 lower littoral zone in Rakkfjorden in July and August the year before.

653 No significant differences in TBARS levels were observed across sites (Fig. 9A). TBARS
654 levels did not differ between genders or across reproductive stages at any site.

655 CAT activity was not different across sites (Fig. 9B), between genders at any site, or
656 across reproductive stages at any site.

657 GST activity was highest in mussels from Rakkfjorden (31.3±12.7 nmol/min/mg protein)
658 (Fig. 9C), however these results were only based on two male individuals, as all the other
659 mussels from this site showed GST activity below the assay's limit of detection. No significant
660 differences in GST activity were found between genders or across reproductive stages at any
661 site.

662



663

664 **Figure 9.** Biomarkers measured in digestive glands of the mussels (n=15 per site) collected in six
665 sites of the spatial study: (A) Lipid peroxidation measured as thiobarbituric reactive substances
666 (TBARS); (B) Catalase (CAT) activity; (C) Glutathione S-transferase (GST) activity. Plots represent
667 the median (line), 25%-75% percentiles (box), min-max range (whisker) and outliers (dots). Letters
668 (a, b) indicate significant differences (ANOVA, $p \leq 0.05$) across sites, with TBARS and CAT activity
669 being not significantly different across sites.

670

671 ## 4. Discussion

672 ### 4.1 Seasonal study

673 The present study provides a relatively large dataset on the baseline levels of commonly
674 used biomarkers (TBARS, CAT, GST, TOSC and LMS) in Arctic *Mytilus* spp. and their
675 variations across seasons. In addition, it gives a description of the annual reproductive cycle of
676 *Mytilus* spp. and their morphological and physiological features related to the location within
677 the tidal zone. The obtained knowledge is important for determination of the natural range of
678 baseline biomarker variability at different seasons and tidal conditions. Moreover, when
679 assessing the acute exposure and following effects of a pollution event, the biomarker results
680 should be adjusted to the pollution history of the considered site and baseline biomarker levels
681 in the same month/season as the incident occurred. This will help to draw a picture as correct as
682 possible of the environmental situation and help decision making during the recovery process.

683 Spawning period in Rakkfjorden took place between June and September, as individuals
684 at stage 0 appeared among collected mussels. It is widely known that the reproduction of the
685 mussels is closely associated with the water temperature (Chipperfield, 1953; Okaniwa et al.,
686 2010). The temperature range between 9.5 and 12.5 °C triggers mussels to start spawning
687 (Chipperfield, 1953). The water temperature in Rakkfjorden was 10 to 14 °C, when mussels
688 spawned. As for the relationship between the highest concentration of phytoplankton in water
689 and start of spawning, it is reported to vary (Thorarinsdóttir et al., 2013). In Rakkfjorden, the
690 Chl A-bloom was observed in March, some months before spawning. The mussels were
691 provided with food from the beginning of March to the end of September. In Iceland, some *M.*
692 *edulis* spawned during the phytoplankton bloom, however, some of the mussels could start
693 reproduction two weeks before the bloom (Thorarinsdóttir et al., 2013).

694 Spawning in mussels from Rakkfjorden was partial, no fully spent individuals were
695 present in the samples in June and July and some mussels were at stages I and II in July and
696 August. These mussels seemed to save some energy to mature for a secondary spawning in
697 August and September, similar to the mussels from Western Norway (Duinker et al., 2008).
698 However, the environmental conditions and food availability were likely not favorable for the
699 secondary spawning event in Rakkfjorden as a few males with mature gonads (stage III) were

700 observed in January. Their gametes would have to be resorbed, as this phenomenon is quite
701 frequent in mussels (Pipe, 1987; Suárez et al., 2005; Thorarinsdóttir et al., 2013).
702 Gametogenesis in mussels from Rakkfjorden started when water temperature became
703 lower (Thorarinsdóttir et al., 2013) and occurred throughout winter from November to March.
704 In this period no individuals at stage 0 were observed. Fully matured gonads were developed by
705 early spring, similarly to the known reproductive development of the *Mytilus spp.* from more
706 temperate regions and, first described by Seed (1969) in British waters.
707 The sampling site in Rakkfjorden was considered to be a good reference site as the
708 bioaccumulated levels of contaminants (PAHs and metals) in mussels there were reported to be
709 low (Nahrgang et al., 2013), with the site characterized as insignificantly polluted (Class I –
710 Background levels; TA-1467/1997).
711 The baseline levels of TBARS in mussels from Rakkfjorden were in the same range as in
712 *Mytilus* spp. from a pristine location in Ireland (Schmidt et al., 2012; Schmidt et al., 2013) and
713 the control *M. galloprovincialis* from an unpolluted site in North West Spain (González-
714 Fernández et al., 2016). The range of CAT in the present study was similar to CAT measured in
715 *M. edulis* from the northern Baltic Sea (Leiniö and Lehtonen, 2005) and the control *M.*
716 *galloprovincialis* from an unpolluted site in North West Spain (González-Fernández et al.,
717 2016). GST levels were also in the same range as in González-Fernández et al. (2016),
718 however, they were lower than reported in Leiniö and Lehtonen (2005); Nahrgang et al. (2013)
719 and Schmidt et al. (2013). The range of TOSC levels and the seasonal trend from this study
720 were similar to the study by Nahrgang et al. (2013).
721 According to the results of the PCA models, reproductive stage/maturity phase of the
722 mussels was found to be one of the most important drivers of the baseline levels of the studied
723 biomarkers. Spawning during spring time in mussels from Rakkfjorden was associated with
724 high levels of oxidative stress biomarkers and LMS. Indeed, CAT activity was high prior to
725 spawning in April 2013; highest levels of TBARS and TOCS-OH were measured in ripe
726 mussels that started to spawn in May; the levels of LMS stayed high during the whole
727 spawning period. High levels of TBARS, CAT and TOSC associated with spawning during
728 spring time were also reported in González-Fernández et al. (2016); Schmidt et al. (2013) and
729 Bocchetti and Regoli (2006), however, Hagger et al. (2010) showed a sharp decrease in LMS
730 coincided with mussel spawning activity.

731 During the period of gonadal restoration after spawning, *Mytilus spp.* from Rakkfjorden
732 tended to have decreased activities of antioxidant enzymes (TBARS, CAT and TOSC) and
733 remain constant during the rest of the year. This indicated that the mussels were likely to be
734 more sensitive to oxidative stress during gonadal development and gametogenesis in autumn
735 and winter. In this period, mussels invested all their energy in reproduction. Also, such
736 physiological state may have led to the lower levels of LMS and higher destabilisation of the
737 lysosome membranes. It remains unclear to what extent the baseline levels of the biomarkers in
738 the present paper were responding to the reproductive state of the mussels and to environmental
739 factors (water temperature and food availability), or a combination of both. For example, the
740 study on *M. edulis* from the Barents Sea reported that the lower the water temperature, the more
741 destabilized were the membranes of lysosomes in haemocytes of the mussels (Camus et al.,
742 2000). The reduction in LMS in *Mytilus* spp. were later explained by an increased physiological
743 stress due to low food availability and intense energy demand of gonadal development during
744 winter time (Nahrgang et al., 2013; Hagger et al., 2010; Balbi et al., 2017). Spawning in
745 Rakkfjorden happened at the same time when water temperature was increased and
746 phytoplankton as a food source for mussels was abundant. In the multivariate statistical
747 analyses used in the present paper, all environmental factors resulted in the poor models and
748 consequently were found to be difficult to use for explaining ecological aspects behind
749 biomarkers and what they really responded to.
750 Tidal zone as a factor strongly influencing the biomarker levels of the mussels from
751 Rakkfjorden was also reported in the results of the multivariate statistics run for the present
752 data. Indeed, the differences in physical conditions of the tidal zones within the mussel bed
753 (e.g. wave energy, sand particle size, desiccation, light, temperature) are known to affect
754 morphology of the mussel shell (MacLachlan et al., 1995) and physiology of the mussels
755 (Altieri, 2006; Petes et al., 2008; Tagliarolo et al., 2012). The air and water temperature logs in
756 Rakkfjorden over the year indicated that mussels in the upper littoral zone were exposed to
757 higher variations of the air temperature (from –10 to +16 °C) than mussels at the lower littoral
758 zone that were in water most of the time (from 0 to +12 °C). Also, the access to food was
759 reduced on the upper littoral zone due to tidal range. Due to natural differences in temperature,
760 time to air exposure and food availability between upper and lower littoral zones, mussels
761 inhabiting them were different in size and CI. The largest mussels in the lower littoral zone had

762 higher volume and higher CI than the largest individuals in the upper littoral zone. In the west
763 coast of Ireland, the larger CI was also reported in *Mytilus* spp. from the low shore compared to
764 the high shore, and this difference was mainly linked to a shorter feeding time on the high shore
765 (Schmidt et al., 2012). It was also observed that shells of the mussels on the upper littoral zone
766 were more rounded than in mussels on the lower littoral zone having the shape of an egg, or
767 ovoid. Such morphological differences might be associated with adaptation of the mussels to
768 keep water inside during low tides and protect their soft tissues from drying (Steffani and
769 Branch, 2003). As for the physiological adaptations, mussels from the upper littoral zone
770 seemed to spawn later than mussels from the lower littoral zone. These differences between
771 tidal zones were possibly a result of the different degree of environmental stress experienced by
772 the mussels. According to Petes et al. (2008), mussels in the upper littoral zone had to invest all
773 their resources to face increased stress due to harsher environmental conditions, and thus were
774 not able to invest as much energy on reproduction as mussels in the lower littoral zone.
775 Taking into account the morphological and physiological differences in mussels at the
776 different tidal zones, the baseline biomarker levels in the mussels across tidal zones were
777 expected to be different too. However, no differences in LMS were found between the tidal
778 zones in Rakkfjorden. The LMS was found to be reduced in the subtidal mussels than in
779 intertidal mussels from the German bight of the North Sea in spring, because subtidal mussels
780 reached maturity during winter and spring consuming higher energy for the reproductive
781 process, whereas intertidal mussels stayed at the resting stage the whole winter and rapidly
782 developed their gonads in spring in order to be able to spawn in summer (Brenner et al., 2014).
783 Also, the present data indicated only few sporadic differences in the biomarkers for antioxidant
784 defense between the tidal zones. GST activity was higher in mussels from the upper littoral
785 zone than from the lower littoral zone in January, suggesting a need for increased antioxidant
786 protection in more stressful habitats of the upper littoral zone (Letendre et al., 2008; Schmidt et
787 al., 2012). Indeed, the difference between water and air temperature was highest in January, the
788 coldest month in the studied year (Fig. S2). However, in contrast to higher CAT activity
789 (Letendre et al., 2008) and higher TBARS levels (Schmidt et al., 2012) on the high shore
790 compared to the low shore, the mussels from Rakkfjorden had higher CAT activity in lower
791 littoral zone than in the upper littoral zone in November, while TBARS levels were similar in
792 both tidal zones.

793 Even though the present study obtained quite detailed seasonal data on both abiotic and
794 biotic factors that might have influenced the baseline biomarker levels of the mussels, the
795 interpretation of the overall results was challenging. The number of collected samples (n=9–12
796 per month from each tidal zone, Table S1) seemed not to be optimal as the individuals within
797 the same sample group varied in terms of gender and reproduction stage. A certain variance in
798 the baseline biomarker levels could also possibly be due to different species or hybrids
799 (Mathiesen et al., 2016). The number of mussels per tidal zone per sampling month could be
800 increased to 20-30 to minimise this variation among individuals, and to increase n for statistical
801 analyses.
802 The collected mussels were smaller in size than the mussels from the same location that
803 were studied by Nahrgang et al. (2013). Indeed, the mean (±SE) length of the mussels in the
804 upper littoral zone was 34.0±0.3 mm and in the lower littoral zone it was 40.9±0.6 mm (Table
805 S2), while the length of the mussels reported in Nahrgang et al. (2013) ranged between 45 and
806 51 mm. Thus, the soft tissues especially after spawning period and during the winter time were
807 not always enough to carry out all the biomarker analyses. This resulted in many gaps in the
808 dataset and consequently in poor statistical models.
809 Concerning the assessment of LMS in living haemocytes using the neutral red retention
810 assay (Martínez-Gómez et al., 2015), the validity of the seasonal results showing the reduction
811 in LMS in *Mytilus* spp. in the winter half-year (Fig. 4) may be questioned. Indeed, the assay,
812 that followed standardized protocols of ICES (Martínez-Gómez et al., 2015) was run in an ice-
813 water cooled chamber placed in the laboratory (ambient air temperature around 22 °C
814 throughout the year). The average air temperature inside the chamber was 17.7±0.3 °C while
815 performing all the assays. However, both water and air temperatures of the mussel environment
816 varied significantly during the year. Mussels are ectothermic animals with ambient temperature
817 being the most important factor influencing their physiological mechanisms and ecology (Huey
818 and Stevenson, 1979; Angilletta et al., 2002). During the assay, the cells were exposed by
819 fluctuating temperature both inside the chamber due to multiple door openings, and on the
820 microscope slides that were taken out of the chamber for frequent evaluation under the light of
821 the microscope, even when a slide check took no more than 10 seconds. Thus, the temperature
822 conditions of the assay, due to fluctuations and levels far higher than ambient temperature,
823 might invalidate the LMS data, especially in November, December and March, when the

824 average LMS levels were the lowest. The haemocytes of the mussels collected in these months
825 likely experienced large temperature jumps (from water temperature of 6, 3 and 3 °C,
826 respectively, to at least 17.7±0.3 °C inside the chamber) that could have caused a potential
827 destabilisation of their membranes during the assay´s conditions. In the experiments on thermal
828 stress on *M. edulis*, the destabilisation of the lysosome membranes in the digestive cells were
829 reported to be induced at 13 °C over ambient temperature (Moore, 1976). Hence, the neutral
830 red retention assay protocol needs to be further refined. A suggestions would be to evaluate the
831 LMS at the mussels' ambient temperature by performing the assay in a climate room.
832 Otherwise, an alternative protocol for LMS in frozen tissue sections (e.g. enzyme cytochemical
833 method; see more in Martínez-Gómez et al., 2015) should be used. In contrast to the results
834 obtained in the present study, measurement of the LMS in *M. galloprovincialis* from the
835 northern coast of Spain by method for N-Acetyl-ß-hexosaminidase indicated that lysosome
836 membranes in the digestive cells were more destabilised in summer than in winter due to the
837 higher feeding activity in summer (Izagirre et al., 2008). Also, LMS in these lysosomes was
838 found to be dependent on the digestion and amount of time mussels were air exposed during the
839 tidal drawdown, and not on the ambient temperature. Thus, the verification of the neutral red
840 retention assay is highly needed, as it could influence the results of monitoring and false the
841 interpretation of monitoring data.
842
843 4.2 Spatial study
844 The biomarker data obtained in the site of the seasonal study in Rakkfjorden was
845 compared with five other sites within a 60-km radius (Fig. 1) in order to determine how
846 applicable the data from the Rakkfjorden site was within the region. Two of the sites (Tromvik
847 and Brensholmen) were similar to Rakkfjorden in terms of the environmental factors (water
848 temperature, salinity and Chl A). The site in Buktalandet differed from Rakkfjorden only in
849 reduced salinity. Two other sites (Simaviknes and Oldervik) were characterized by lower water
850 temperature, lower salinity (Oldervik) and higher Chl A (Simaviknes) compared to
851 Rakkfjorden. The individual parameters of the mussels (shell volume, sex ratio and distribution
852 of the reproductive stages) were similar in Rakkfjorden and Buktalandet. Mussels from
853 Tromvik, Brensholmen and Oldervik were larger in shell volume than mussels from
854 Rakkfjorden. In addition, most of the mussels from Tromvik were females, while more males

855 were presented in the samples from Rakkfjorden. Most of the mussels from Simaviknes were in
856 the late maturity phase and seemed to spawn later compared to the mussels from all other sites
857 that were either in the resting stage 0 or had just started gametogenesis (stage I).

858 The levels of bioaccumulated PAHs in the mussel soft tissues indicated that mussels at all
859 sites were moderately (Class II – No toxic effects) polluted (TA-1467/1997). Concentrations of
860 metals at all sites were either at moderate (Class II – No toxic effects) or at marked (Class III –
861 Chronic effects) levels. None of the mussels were severely (Class IV – Acute toxic effects) or
862 extremely (Class V – Severe acute toxic effects) contaminated by pollutants. Thus, all sites of
863 the spatial study likely represented a current environmental quality status in the region affected
864 by increases in maritime traffic during the past decade (Dybedal et al., 2015; Borch et al.,
865 2016).

866 The bioaccumulated concentrations of PAHs and metals in the soft tissues of mussels
867 from Rakkfjorden increased from being insignificant (Class I – Background levels of PAHs,
868 Cd, Cu, Hg, Pb, Zn) and moderate (Class II – No toxic effects of As) in June 2010 (Nahrgang
869 et al., 2013) to moderate (Class II – No toxic effects of PAHs, Hg, Pb) and marked (Class III –
870 Chronic effects of As, Cd, Cu, Zn) measured in the present study in July 2014. The
871 concentrations of Cr and Ni, however, became lower during these four years, but they still fell
872 into the moderate degree of pollution (Class II – No toxic effects). For now, it is difficult to
873 conclude that such increase in bioaccumulated levels of PAHs and metals in mussels from
874 Rakkfjorden was a result of the increased pollution in the region. It is also known that there
875 could be yearly variations in bioaccumulation due to different intensity of the phytoplankton
876 blooms and inter-annual shift of the gametogenesis period (Fattorini et al., 2008). In addition,
877 seasonal variations in lipid content in the soft tissues of the mussels and changes in food quality
878 and quantity were reported to have an effect on the bioaccumulation in mussels (Nahrgang et
879 al., 2013). Thus, several inter-annual measurements of bioaccumulated levels should be done to
880 verify whether there truly is an increasing trend, or whether this is within a normal range of
881 annual and seasonal fluctuations.

882 The variations in the environmental factors (water temperature, salinity and Chl A
883 concentration in water) across the sites, few sporadic differences in the individual parameters of
884 mussels (size, sex ratio and distribution of the reproductive stages) and their different
885 bioaccumulation levels of NPDs, Cd, Cr, Cu, Pb, Zn were not reflected in the baseline levels of

886 biomarkers (TBARS, CAT and GST; Fig. 9). The lack of differences in biomarker responses to
887 oxidative stress and pollutant levels measured in mussels between Rakkfjorden and five sites
888 within a 60-km radius from it suggested that mussels from Rakkfjorden could be representative
889 for mussel populations on the North Norwegian coast, or at least within the Tromsø region.
890

891 ## 5. Conclusion

892 The design of the present study was based on the ICES guidelines (ICES, 2010) and
893 practical recommendations given for the biomonitoring of the Arctic *Mytilus* spp. in Nahrgang
894 et al. (2013). The present study examined baseline levels of commonly used pollutant
895 biomarkers in *Mytilus* spp. over the course of a year and indicated some exogenous and
896 endogenous factors that could potentially affect these levels and provide a natural range of their
897 variability. Moreover, mapping spatial baseline variations in biomarkers of the mussel
898 populations from different tidal zones and different sampling sites within the Tromsø region on
899 the North Norwegian coast contributed to the improvement of our ability to use mussels as
900 sentinel species for environmental monitoring purposes in Arctic environments. The
901 morphological, histological and biomarker data obtained for the studied mussels, as well as the
902 logging of the environmental parameters, provided relevant baseline information and may be
903 useful in future biomonitoring studies of the present region.

904 Combination of the data obtained in the present study and study by Nahrgang et al.
905 (2013), as well as recommendations for future improvement of the present study in terms of
906 optimal number of mussels per sampling, careful selection of the mussels large enough to carry
907 out the biomarker analyses, measurement of their chemical bioaccumulation over several years
908 and reassessment of the LMS adjusted to the ambient temperature of the mussels will definitely
909 provide a thorough database for biomonitoring procedures in the Arctic regions.
910

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Conflict of interest

The authors have no conflicts of interest to declare.

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