1   # Ecosystem metabolism of benthic and pelagic zones of a shallow productive

2   ## estuary: Spatio-temporal variability

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Abstract

Long-term deterioration of water quality is known to reduce the relative importance of benthic to pelagic ecosystem metabolism in shallow coastal ecosystems, but drivers of spatial and short-term variability in ecosystem metabolism are poorly understood. We address this knowledge gap through detailed seasonal measurements of ecosystem metabolism across depth gradients from shallow (2–3 m), eelgrass-dominated to deeper (4–5 m), muddy regions of a shallow, productive estuary. Combined measurements of gross primary production (GPP), respiration (R) and, by difference, net ecosystem production (NEP) by the open water diel oxygen technique and *in-situ* chamber incubations showed high importance of shallow eelgrass habitats for metabolism at the system scale. Seasonal variations in GPP, R and NEP increased with light availability and temperature with highest NEP in all habitats during the warm and sunny mid-summer. The shallow eelgrass-dominated and neighboring habitats were seasonally net autotrophic (NEP = 0.54 and 0.31 mg O_2 m^{-2} d^{-1}), compared to net heterotrophy (NEP = -0.26 mg O_2 m^{-2} d^{-1}) at the deeper, muddy site. Detailed studies along depth gradients further confirmed the role of eelgrass as a key driver of spatial differences in ecosystem metabolism across the estuary. Strong northerly winds (>8 m s^{-1}) caused short-term (< 24 h) periods of similar oxygen dynamics and similar apparent productivity in shallow and deeper waters, indicative of efficient lateral mixing while calm periods (< 4 m s^{-1}) enabled formation of "pockets", i.e. water masses with limited connectivity, which exacerbated the metabolic differences between shallow and deep sites.

Introduction

Benthic primary producers such as seagrasses play important ecological roles as hotspots for production, storage and export of organic carbon (Duarte et al. 2013, Duarte 2017) in addition to

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43 efficiently retaining nutrients, stabilizing sediments and stimulating biodiversity in shallow

44 coastal ecosystems (e.g. Hemminga & Duarte 2000). The relative importance of benthic and

45 pelagic primary producers in shallow estuaries is largely a function of water clarity, and strongly

46 related to nutrient loading (Borum & Sand-Jensen 1996). Shallow coastal seafloors typically

47 have a high cover of benthic macrophytes in the well-lit photic zone, where they can contribute

48 significantly to gross primary production (Duarte & Chiscano 1999, McGlathery et al. 2001,

49 Öberg 2006). Reductions in water clarity of shallow coastal waters, mostly due to eutrophication,

50 have caused global losses and reduced depth colonization of seagrass meadows (Short & Wyllie-

51 Echeverria 1996, Orth et al. 2006, Waycott et al. 2009). Historically, most of the Danish

52 estuaries were dominated by the seagrass *Zostera marina* (eelgrass), but following the wasting

53 disease in the 1930s, and partial recovery thereafter, the extent and depth distribution of eelgrass

54 decreased markedly, as eutrophication reduced water clarity (Nielsen et al. 1992, Boström et al.

55 2014). For example, Limfjorden in Denmark experienced a shift from a pristine, benthic

56 macrophyte-dominated clear water regime with high total gross primary production (GPP) in the

57 early twentieth century to an eutrophic, plankton-dominated regime still with high total GPP in

58 the 1980s when nutrient loadings peaked (Krause-Jensen et al. 2012). Nutrient loadings and

59 concentrations in Danish coastal waters have decreased significantly since the 1980s, but

60 improvements in water clarity and eelgrass depth colonization have been slow with only recent

61 signs of recovery process starting (Riemann et al. 2016). While the eutrophication-induced

62 transition from a benthic to a pelagic dominated system is well documented, the reversal –

63 oligotrophication is less well understood (Duarte et al. 2009, Riemann et al. 2016), partly

64 because of multiple feedback mechanisms, including unsuitable soft and organic-rich substratum,

65 that potentially delay the return of the vegetation (Maxwell et al. 2017). A few recent studies,

66   however, present trajectories of recovery, and changes in overall productivity with a reversal to a

67   benthic dominated system (McGlathery et al. 2012, Riemann et al. 2016, Staehr et al. 2017).

68   In shallow coastal ecosystems such as Roskilde Fjord, Denmark, where sufficient light reaches

69   the sediment surface to support benthic primary production in most of the estuary (Staehr &

70   Borum 2011), net ecosystem production (NEP) is expected to be autotrophic (NEP > 0) with

71   GPP exceeding ecosystem respiration (R) (Ziegler & Benner 1998, Kemp & Testa 2011). This

72   expectation agrees with observed long-term improvements in water clarity and greater depth

73   limits of the dominant macrophyte, *Zostera marina,* associated with increasingly autotrophic

74   conditions (Staehr et al. 2017). Although several studies have assessed the relative roles of

75   pelagic and benthic compartments in shallow aquatic ecosystems (e.g. Borum & Sand-Jensen

76   1996, Krause-Jensen et al. 2012, Van de Bogert et al. 2007), only few studies have allowed a

77   direct partitioning of the metabolic processes (e.g. Caffrey et al. 1998, Hume et al. 2011, Murrell

78   et al. 2017). Moreover, the relative contribution of benthic versus pelagic primary producers

79   along depth gradients and the temporal dynamics in ecosystem metabolism are poorly

80   understood (Kamp-Nielsen 1992, Kemp & Testa 2011).

81   Moving from shallow well-illuminated to deeper shaded habitats will likely shift net ecosystem

82   production from autotrophy to heterotrophy with increasing plankton respiration and decreasing

83   benthic photosynthesis. In support of this, daily variation of oxygen and temperature show

84   declines with depth, indicative of higher metabolic rates in the near-shore shallower waters

85   related to the presence of rooted autotrophs (Odum 1967, Swaney 1999). The partitioning of total

86   ecosystem respiration between planktonic and benthic components is also expected to change

87   with depth, as planktonic processes are strongly favored in deeper systems (e.g., Kemp et

88   al.1992, Heip et al. 1995). Moreover, temporal variation in external forcing variables, including

4

89 sunlight, wind, meteorological tides, and stream flow may cause significant day-to-day, and even

90 hourly variability in primary production, respiration, and net ecosystem production (Jennings et

91 al. 2012, Staehr & Sand-Jensen 2007). Short-term hourly increases in wind-induced water

92 turbulence can, hence, lead to higher rates of sediment resuspension, reducing water clarity, light

93 penetration, and photosynthesis (Arfi et al. 1993) as well as reintroduction of labile organic

94 matter into the water column, stimulating planktonic respiration (Demers et al. 1987). Large

95 daily variability in ecosystem production has been associated with variations in cloud cover and

96 the resulting changes in sunlight (e.g., Fisher et al. 2003), as well as with wind-induced changes

97 in mixing and resuspension (Staehr & Sand-Jensen 2007). Depending on water depth, sediment

98 characteristics and density of macrophytes, such external forcing will interact with the complex

99 local hydrodynamics associated with the canopy structure, eventually influencing the oxygen

100 dynamics and metabolic processes in nearshore shallow habitats differently (Hume et al. 2011).

101 In this study, we utilized high-frequency open-water measurements of oxygen, temperature,

102 salinity and wind speed to investigate temporal and spatial variability in estuarine metabolism at

103 sites with different benthic habitats and at different water depth. This enabled us to characterize

104 the seasonality in GPP, R and NEP and investigate the regulatory importance of different

105 external conditions over different time scales. A series of benthic and pelagic oxygen *in-situ*

106 incubation studies furthermore allowed assessment of the benthic and pelagic contributions to the

107 ecosystem-integrated rates. Multiple measurements along a depth gradient made it possible to

108 test expectations of larger magnitude of variability of ecosystem metabolism in shallow eelgrass-

109 dominated areas compared to deeper muddy parts of the estuary. These measurements along a

110 depth gradient ultimately enabled us to evaluate the influence of meteorological forcing events

111 for short-term oxygen dynamics in the coastal environment.

Methods and materials

Study sites

Our study was conducted in the southern, inner part of Roskilde Fjord, a 30 km long, shallow

estuary (mean depth 3 m, surface area 123 km^2) in north Zealand, Denmark (Fig. 1). The outer

part connects to the Kattegat through the Isefjord, while a sill restricts water transport between

the outer and inner part of the estuary. The inner part is slightly shallower than the outer but has

a larger surface area, which overall results in 23% larger volume. The water column in the inner

part of the estuary is well-mixed, with only short sub-diel stratification. Depending on the

strength of wind driven exchanges, the average water residence time in the inner part of the

estuary ranges between 90 and 720 days (Kamp-Nielsen 1992, Josefson & Rasmussen 2000).

Land use in the catchment is dominated by agriculture (67%) whereas urban areas account for

15% and the remaining watershed is covered with forests, wetlands, and lakes. Roskilde, the

largest city in the catchment, lies on the shore of the inner basin. Historically, Roskilde Fjord,

like the rest of Denmark, had very extended eelgrass meadows (Petersen 1901, Steemann-

Nielsen 1951), and while these were severely reduced by eutrophication, peaking in the 1980s,

Roskilde Fjord and Denmark are still hotspots of eelgrass distribution and experiencing recent

increases in eelgrass depth distribution (Riemann et al. 2016, Staehr et al. 2017).

Temporal variability in ecosystem metabolism was investigated through continued open system

diel O$_2$ measurements as well as through episodic benthic and pelagic incubations in three main

sites in the inner part of Roskilde Fjord from April (spring) to December (winter) 2015 (Fig. 1,

Table 1). Two of the sites were located at 2-3 m depth, one representing an eelgrass meadow

("Eelgrass") and another, approximately 100 m to the south, representing a neighboring non-

135 eelgrass site. The latter is referred to as the "Bare" site, although the site had scattered small

136 stones (5 – 10 cm in diameter) covered by filamentous algae. The third site ("Muddy")

137 represented the deeper (5 m) area characterized by fluffy organic-rich sediment with no benthic

138 vegetation. In our study, we distinguish between eelgrass dominated vs. bare vs. mud dominated

139 areas and refer to these as different habitats due to their marked differences in substrate and

140 vegetation. Furthermore, to quantify short-term effects of physical forcing, we conducted a

141 gradient study in May 2016 along a transect perpendicular to the shoreline (from ~100 to 500 m

142 off the shore) starting at 1.8 m and reaching ~5 m depth and representing all three habitat types

143 (Table 1, Fig. 1).

144 *Water quality sampling*

145 Water quality sampling for measurements of total and dissolved inorganic nutrients,

146 phytoplankton biomass (chlorophyll a; Chl *a*), Secchi depth and profiles of temperature, salinity

147 and oxygen was carried out every two weeks from May to December 2015 at the central muddy

148 station, which coincided with a monitoring site (Fig. 1; St. 60) within the Danish National

149 Aquatic Monitoring and Assessment Program (DNAMAP). Sampling and measurements of

150 dissolved inorganic nitrogen (DIN; $NO_3^- + NO_2^- + NH_4^+$), dissolved inorganic phosphorus (DIP;

151 PO_4^{3-}) and Chl *a*, followed standard technical guidelines (Kaas & Markager 1998). Water

152 column concentrations of nutrients were measured at 1 m depth by colorimetric methods (Danish

153 Standards Association, 1975, 1985). Chl *a* was analyzed from Whatman GF/F or GF 75

154 Advantec filtered samples from 1 m depths and extracted in ethanol according to Strickland and

155 Parsons (1972). To supplement these monitoring data, water samples were taken from 1 m depth

156 on a biweekly to monthly basis to measure pelagic primary production using a modified oxygen

157 incubation technique as described below.

158 *Field measurements of metabolic rates and physical forcing*

159 <u>Diel oxygen measurements</u>: Rates GPPR and NEP were determined from changes in the

160 concentration of dissolved oxygen (DO) following the diel oxygen technique as originally

161 described by Odum (1956). In our study, continuous measurements (every 10 min) of DO and

162 water temperature were made with miniDOT oxygen optode loggers (Precision Measurement

163 Engineering, Vista, CA, USA, in the following termed "oxygen logger") at the three main sites.

164 Photosynthetically active radiation (PAR) was measured simultaneously at the same frequency

165 using four Odyssey PAR loggers (Dataflow Systems Pty Limited, henceforth termed "PAR

166 loggers") placed within a 2 meter depth range at all three sites. Odyssey loggers were calibrated

167 based on parallel measurements of PAR by a LICOR® 2π sensor. All sensors were cleaned on a

168 bi-weekly basis to reduce sensor drift from fouling. Fouling of sensors was further reduced by a

169 copper mesh for the oxygen sensors and by a copper film placed around the light sensors. Sensor

170 drift was corrected by assuming a linear decline in sensitivity over each bi-weekly period.

171 From April to December 2015, we applied two oxygen loggers and a PAR logger at each site

172 from April to December 2015 (Table 1). At the deeper muddy site, oxygen loggers were attached

173 to a surface buoy approximately one meter below the sea surface while at the shallow sites

174 sensors were fixed to metal poles secured firmly into the sediment, measuring approximately 30

175 cm above the seafloor. We do not anticipate any effects of sonde placement above sediment as

176 the water column was fully mixed at all three sites throughout the measurement period. At each

177 site we calculated the diffuse light attenuation coefficient (K_{PAR}) as the linear slope between

178 depth and the log of light intensity. Regressions with a poorer fit ($r^2 < 0.8$) were excluded from

179 further analysis.

180 The supplementary short-term study of diel O_2 variability represented a four-week period from

181 26th of April to 20th of May 2016 (Table 1), we deployed five oxygen loggers, and one YSI

182 6600V2® multisonde measuring oxygen, temperature, water level and salinity. The equipment

183 was placed along a depth gradient from 1.8 m inside the eelgrass meadow extending

184 approximately 500 m perpendicular to the shoreline, northwest towards the muddy site at 5 m

185 depth. All sensors were attached to metal poles and positioned ~30 cm above the sediment

186 surface.

187 Benthic incubations: To investigate the importance of eelgrass meadows and other benthic

188 components for diel variations in oxygen concentrations and daily water column net fluxes, we

189 used *in-situ* chambers, which allowed us to incubate a section of the seafloor with its benthic

190 community. Triplicate chamber incubations were done at the three types of habitat in April

191 (spring), June (early summer) and August (mid/late summer) 2015, corresponding to mean

192 temperatures of 9, 16 and 21°C, respectively. Chambers consisted of gas impermeable

193 transparent plastic bags (19 x 42 cm; diameter x height = 12 L) attached to a hard PVC collar,

194 which was secured firmly into the sediment with metal plugs (Fig. 2A). Incubations were made

195 over a period of approximately 24 hours with logging of oxygen, temperature and light every 10

196 minutes by an oxygen logger and a light logger placed at a depth of about 30 cm above the

197 seafloor inside each chamber fixed to a metal stick placed into the sediment.

198 Pelagic incubations: Water samples were collected on 14 occasions from April to December at 1-

199 2 m depth at the deeper muddy site. These pelagic rates were assumed to also represent pelagic

200 rates at the shallower bare and eelgrass sites. Triplicate measurements of pelagic metabolism

201 were done by *in-situ* incubation of the sampled water in 0.5 L transparent glass jars attached to a

202 buoy over approximate 24 hours at 1 m depth. An oxygen logger was inserted 5 cm into each

9

203   glass jar and recorded oxygen and temperature every 10 minutes. Next to these jars, a PAR

204   logger recorded light simultaneously within the same frequency.

205   *Modelling of oxygen fluxes*

206   To improve comparisons of metabolic rates between open-water and chamber measurements, we

207   applied a similar inverse modeling approach (Hanson et al. 2008, Brigenthi et al. 2015), which

208   utilizes data on irradiance and temperature to model metabolic rates from high-frequency oxygen

209   measurements. This approach has less assumptions regarding constant respiration and facilitated

210   more realistic daily rates using information on the ambient light and temperature conditions during

211   similar periods. This approach minimizes possible mismatch between open-water and chamber

212   fluxes that may result from differences in light and temperature conditions during sampling.

213

214   Diel changes in DO for open-water measurements, pelagic bottle incubations and benthic

215   chambers were modelled using equation 1:

216 $$DO_{[t+1]} = DO_{[t]} + GPP_{[t]} - R_{[t]} + F_{[t]} \qquad\qquad (1)$$

217   where $DO_{[t+1]}$ and $DO_{[t]}$ are the DO concentrations (mg L^{-1}) at discrete times $t + 1$ and t with 10

218   min resolution; $GPP_{[t]}$ is the gross primary production at time t; $R_{[t]}$ is the ecosystem respiration at

219   time t (eq. 2); and $F_{[t]}$ is the net exchange of O_2 between the lake and the atmosphere at time t (eq.

220   3). For bottle and chamber incubations with no air-water gas exchange, eq.1 was simplified by

221   removing the $F_{[t]}$ term. Net ecosystem production (NEP) was calculated using a light and

222   temperature dependent model (eq. 2) described by Brighenti et al. (2015):

223 $$\Delta DO_{t+1} = NEP_{hr} = P_{max} \times tanh(\alpha \times I_t / P_{max}) - R_{20} \times 1.07^{T_t - 20} \qquad\qquad (2)$$

224 where P_{max} is the light saturation point, α is the initial linear slope of the photosynthesis vs. light

225 relationship describing the average rate of photosynthesis per unit of PAR, I_t is the surface PAR

226 (µmol photons m^{-2} s^{-1}) measured at time t. Respiration was a function of the rate of R at 20℃,

227 water temperature (T_t) and a temperature sensitivity constant equal to 1.07.

228 Net oxygen exchange between air and water was calculated according to Staehr et al. (2010):

229 $$F_{[t]} = (k_{[t]} \, (DO_{[t]} - DO_{sat[t]})/Z)/2 \qquad (3)$$

230 where DO is the measured concentration in the water and DO_{sat} is the concentration in water at

231 equilibrium with the atmosphere at ambient temperature and pressure (Weiss 1970) at time t, $k_{[t]}$

232 is the coefficient of gas exchange for DO at time t and Z is the total depth of the water column at

233 the measurement site. k was calculated based on the relationship among Schmidt numbers (Sc)

234 and temperature (Jähne et al. 1987): $k = k_{600} \times (Sc/600)^{-0.5}$, where k_{600} is the piston velocity

235 calculated as function of wind speed at 10 m height according to a model developed for Roskilde

236 Fjord (Mørk et al. 2014). Wind speeds were measured at a meteorology mast 1 km away from the

237 experimental area.

238 The three parameters in equation 2 (P_{max}, α and R_{max}) were estimated using a conjugate-gradient

239 optimization algorithm that minimized the sum of squared errors between estimated and observed

240 DO for each incubation period with constraints on the parameter space, i.e. lower and upper bound

241 for each parameter. The parameters P_{max}, α, and R_{max} were finally used to estimate DO

242 concentrations at every 10 minutes using equation 1 above. Parameterization of DO curves can be

243 poor on days where oxygen dynamics are dominated by physical exchanges (Hanson et al. 2008).

244 To assess model performance (i.e., how well the model fitted the observed DO data), we

245 determined the coefficient of determination (r^2). To reduce bias by erroneously modeled DO

246   curves, we removed sonde days with r^2 below 0.2 (Obrador et al. 2014) resulting in removal of

247   15% of the 1068 sonde days measured in total.

248   The optimized parameters, P_{max}, α and R_{max}, were subsequently used to calculate hourly rates of

249   NEP, GPP and R inserting 10 min interval recordings of the mean available light in the water

250   column and water temperature over a 24 h period. Mean light availability (E_{mean}) for primary

251   producers (*i.e.* the expected value of PAR to which an algal cell is exposed, assuming that the

252   water column is fully mixed) was calculated according to Staehr & Sand-Jensen (2007):

253 $$E_{mean} \ = \ E_0(1 - e^{-K_{PAR}Z})/(K_{PAR}Z) \tag{4}$$

254   where K_{PAR} is the diffuse PAR attenuation coefficient (m^{-1}), Z is the total depth of the water

255   column, and E_0 is the surface PAR (μmol photon m^{-2} s^{-1}) measured at the nearby (1 km)

256   meteorological mast.

257

258   Daily rates of GPP, NEP, and R were calculated as the average hourly rates (over 24 hours)

259   multiplied by 24 hours. Volumetric daily rates were then converted to area-specific rates. For open-

260   water measurements and bottle incubations, this simply involved multiplying volumetric rates (g

261   O_2 m^{-3} d^{-1}) with the depth (m) of the water column at the measurement site. For benthic chamber

262   incubations, this conversion involved multiplying with the volume (12 L on average) of the

263   incubation bags and dividing by the area (0.028 m^2) of the incubation chambers. We display

264   respiration rates as negative values to facilitate rate comparison.

265

266

267 *Statistical analysis*

268 To enable a comparison of open-water (total), pelagic and benthic estimates of GPP, R and NEP,

269 we applied a general linear mixed model (GLMM) describing spatial variation among habitats

270 and seasonal (at a monthly resolution) variation as fixed factors, and variation among sampling

271 days within months as random factors in addition to residual variation. Since data on metabolic

272 rates were not consistently measured throughout the entire study period for all three habitats

273 (muddy, bare and eelgrass), marginal means were calculated from the GLMM to produce

274 comparable means for spatial and seasonal variation. The significance of the fixed effects in the

275 GLMM was tested using an F-test for the additional variation explained by the given factor, i.e.

276 comparing the models with and without the factor. Test for differences in metabolic rates among

277 habitats were performed as contrasts between parameter estimates. The GLMM was analyzed

278 using PROC MIXED in SAS v. 9.3. Furthermore, the short-term temporal variability in GPP, R

279 and NEP for the open-water estimates was estimated by calculating the standard deviation of the

280 residuals between observations and a 15-day moving average across the entire study period.

281 Statistical investigation of relationships between variables was performed using a simple

282 Spearman ranks correlation analysis on theuntransformed data. Finally, to evaluate the level of

283 background respiration (*sensu* Solomon et al. 2013) associated with particulate and dissolved

284 organic matter, we performed a linear regression model of R as a function of GPP (using

285 geometric means), and estimated the background respiration level as the y-intercept.

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Results

Seasonal changes in physical and chemical conditions

Depth profiles showed that the water column was fully mixed from April to December with similar temperature and salinity (data not shown) at surface and bottom at the deeper muddy station (Figure 3A). Daily surface irradiance showed large day-to-day variation and peaked around the equinox in late June, approximately two months before the peak of water temperatures (Figure 3A). Daily wind speeds averaged 4.4 m s^{-1} (range: 1.2 - 9.7 m s^{-1}), with 47% of days having wind speeds <4 m s^{-1} and only 2 % having wind speeds >8 m s^{-1} (Figure 3B). Chl *a* levels were overall low (range: 0.9 to 8.9 µg L^{-1}) and Secchi depths relatively high (range: 3.2 to 5.1 m ~ visible at bottom), considering the rather high nutrient levels (Figure 3C). Secchi depth was strongly related to Chl *a* (r_s = -0.54, *p* = 0.008). Chl *a* peaked in late April indicating a late spring bloom (Figure 3C) with a corresponding drawdown of DIN. Nitrogen remained almost completely depleted until late August when a small increase occurred (Figure 3D). Following a phytoplankton bloom in September (Figure 3C), DIN levels declined. During the DIN-depleted summer period, DIP increased, followed by decreasing concentrations in late autumn as DIN accumulated (Figure 3D).

Temporal pattern of ecosystem metabolism in different habitats

All three habitats exhibited a clear seasonality in open-water estimates of GPP, NEP, and R (Fig. 4; Table 2). Rates of primary production and respiration increased during spring, reaching maximum in late summer and declining in fall and winter (Fig. 4). GPP increased more strongly with water temperatures and solar radiation than R (Table 3). While GPP seemed unrelated to wind at the deepest site, there was a significant correlation between respiration rates and wind speeds for all three habitats. In combination, these relationships indicate that net autotrophy (NEP > 0)

312   prevailed during warm, sunny and calm periods (Table 3). Rates of ecosystem respiration were

313   weakly correlated with GPP in the two shallow habitats (r_s = 0.34, p < 0.001) compared to a

314   stronger correlation in the muddy habitat (r_s = 0.63, p < 0.001). Regression analysis of open water

315   R vs GPP showed that the background respiration level was very low as it was not significantly

316   different from zero (Students t-test, p > 0.05) in any of the habitats.

317

318   There were overall significant differences in open water metabolic rates among the three habitats

319   (Table 2). Areal GPP was significantly lower in the deeper muddy habitat (4.60 ± 0.59 g O_2 m^{-2} d^{-1}

320    $^{-1}$; mean $\pm$ 95%CL) compared to the eelgrass (5.26 ± 0.23 g O_2 m^{-2} d^{-1}) and bare habitats ($5.40 \pm$

321   0.23 g O_2 m^{-2} d^{-1}). Area respiration rates were slightly higher in the bare habitat (5.13 ± 0.33 g O_2

322   m^{-2} d^{-1}) compared to the eelgrass (4.71 ± 0.32 g O_2 m^{-2} d^{-1}) and muddy habitats (4.84 ± 0.66 g O_2

323   m^{-2} d^{-1}). The metabolic balance (NEP = GPP – R) was overall positive for the eelgrass (0.54 ± 0.36

324   g O_2 m^{-2} d^{-1}) and bare habitats (0.31 ± 0.36 g O_2 m^{-2} d^{-1}) but negative for the muddy habitat (-0.26

325    $\pm$ 0.64 g O_2 m^{-2} d^{-1}) supporting expectations of net autotrophy in the vegetated area and net

326   heterotrophy in the non-vegetated deeper muddy area.

327   Days of net heterotrophy (NEP < 0) occurred during all months for all three habitats (Fig. 4), but

328   were slightly more frequent at the muddy site (48%) compared to the shallower sites (44%). While

329   negative NEP occurred in all months, the shallow sites mostly experienced net heterotrophy during

330   autumn, suggesting a significant loss of autotrophic biomass here. The analysis of residuals from

331   the 15-day moving average trend showed higher day-to-day variability in open-water metabolic

332   rates in the muddy habitat, especially for GPP and R (cf. Fig. 4). Standard deviations of the

333   residuals from the muddy habitat were 2.71, 2.67 and 2.74 g O_2 m^{-2} d^{-1} for GPP, R and NEP. In

334   comparison, standard deviations of the residuals in the bare habitat were 1.63, 2.43 and 2.63 g O_2

335   m^{-2} d^{-1}, almost similar to those of the eelgrass habitat (1.65, 2.30 and 2.50 g O_2 m^{-2} d^{-1} for GPP, R

336   and NEP, respectively).

337   *Contribution of pelagic and benthic habitats to ecosystem integrated rates*

338   Based on the bottle incubations, we determined areal-specific rates of pelagic GPP, R and NEP,

339   which were much lower than rates obtained with the open-water measurements and the benthic

340   chamber incubations (Fig. 5). Pelagic primary production for the entire period (April to

341   December) was on average 2.0 g O_2 m^{-2} d^{-1}, compared to pelagic respiration rates of 1.6 g O_2 m^{-2}

342   d^{-1}, indicating that the water column was overall net autotrophic (0.4 g O_2 m^{-2} d^{-1}). Pelagic GPP

343   estimates suggested moderate seasonal variation in productivity (Fig. 6), although the statistical

344   test showed no significant difference between months (Table 2).

345   Benthic production and respiration rates, measured in benthic incubation chambers, were overall

346   much higher in the eelgrass beds compared to the nearby shallow bare sediment and the deeper

347   muddy site (Table 4). The eelgrass site was the only habitat with a net positive flux of oxygen

348   (NEP > 0) from the sediment to the water column (Table 4). Low metabolic rates in the eelgrass

349   habitat in April were measured during strong winds (> 8 m s^{-1}), low irradiance and highly turbid

350   cold water, possibly explaining the much lower rates compared to other months (Fig. 6). Benthic

351   rates of both GPP, R, and NEP all showed significant seasonal variation with significant

352   differences among the three sites (Table 3). For GPP and NEP, there was significant interaction

353   between habitats and seasons, indicating that seasonal variation differed among habitats.

354   Comparing open-water (~total) seasonal average estimates of GPP, R and NEP with estimates

355   based on *in-situ* benthic chambers (~benthic) and bottle incubations (~pelagic), showed a close

356   (~100%) agreement between total and the sum of benthic + pelagic rates for the shallow eelgrass

357 site (Fig. 5). Interestingly, although the bare shallow site had total metabolic rates very similar to

358 the nearby eelgrass site, the sum of the measured benthic and pelagic rates could only account

359 for 37% of the open-water GPP estimates and 54% of the ecosystem respiration rates measured

360 at the bare site (Fig. 5). A similar discrepancy was found for the muddy site where the sum of

361 benthic and pelagic metabolic rates only accounted for 46 and 47% of open-water GPP and R,

362 respectively. This discrepancy between open-water and chamber-based rates for the bare and

363 muddy sites seemed connected to the substantially lower benthic rates measured in these

364 habitats, compared to the eelgrass-dominated habitat where benthic rates were multifold higher

365 (Fig. 5). However, integrating across all months and habitats with the GLMM, total rates of GPP,

366 R and NEP were only approximately 10% higher than the sum of pelagic and benthic rates,

367 suggesting a relative good agreement between estimated rates. While pelagic and benthic

368 processes showed different seasonality, their overall contribution to total rates were similar with

369 54, 56 and 46% of GPP, R and NEP, respectively, accounted for by benthic processes.

370 *Changes in metabolic rates with depth and wind conditions*

371 Deploying an array of oxygen, light and temperature loggers for four weeks from the near-shore

372 shallow (1.8 m) to the deeper central site (5 m), enabled us to calculate daily rates of GPP, R and

373 NEP along a depth gradient (Fig. 7). Although volumetric rates were three-fold higher at the

374 shallow sites, water column-integrated rates were more similar along the gradient. Nevertheless,

375 shallow sites had almost twice as high areal GPP and R rates, with small albeit positive and

376 significant NEP compared to NEP ~ 0 at the deeper sites.

377 As two of our oxygen loggers in the 3 - 4 m depth interval malfunctioned, our characterization of

378 the depth gradient was not as detailed as planned. Aggregating data into a near-shore (1.8 to 2.1 m

379 depth) and deep (4.1 to 5 m depth) area, allowed us to investigate the importance of short-term

380 wind and water movement events for diel changes in oxygen and the derived daily rates of

381 ecosystem metabolism at contrasting depths (Fig. 8). During the four-week measurement

382 campaign, considerable variation in wind speed, wind direction, water level and surface light was

383 observed. The relatively long, narrow and shallow hydromorphology of the estuary, oriented in a

384 south-north direction (Fig. 1), makes water level in the inner part sensitive to northerly winds,

385 forcing water from the outer broad into the shallow inner broad, where our study was carried out.

386 This wind-driven increase in water level was clearly observed on three occasions during our

387 measurement campaign, and the increases in water levels scaled with the duration and strength of

388 northerly winds (Fig. 8AB). In the first event, relatively weak northerly winds (4 to 5 m s^{-1}) only

389 persisted for two days causing a water level rise of 0.3 m (Fig. 8AB). The second event lasted for

390 three days with similarly weak winds, causing a water level rise of 0.4 m. The last event lasted

391 five days but with winds ranging between 6 to 12 m s^{-1}, resulting in a water level rise of 0.5 m

392 lasting for three days (Fig. 8AB). During this last event, observed *in-situ* light levels dropped

393 considerably. Although these meteorological events were short-lived, they had marked effects on

394 the variability and magnitude of DO at the shallow sites (Fig. 8C). Large daily variability in oxygen

395 ranging from 80 to 200 % saturation were immediately reduced to variations similar to the deeper

396 offshore sites with diel oxygen variations ranging between 80 and 110 % saturation (Fig. 8C).

397 Consequently, daily rates of GPP were lower on days with reduced oxygen dynamics, with the

398 duration and strength of the wind-driven water movements driving rates down (Fig. 8D). This

399 indirect effect of wind on apparent ecosystem productivity was also evident when plotting GPP

400 and NEP against daily mean levels of wind speed (Fig. 9). While productivity at the deeper sites

401 appeared to be unaffected by wind conditions, winds had a significant negative effect on both

402 apparent GPP and NEP at the shallow sites becoming net heterotrophic at winds above 8 m s^{-1}.

Discussion

This study provides a direct comparison of integrated ecosystem metabolism rates in three functionally different habitats within a temperate shallow estuary. Seasonal application of the diel oxygen technique in combination with incubations of benthic versus pelagic compartments at different depths furthermore enabled us to evaluate conditions affecting temporal variability along a depth gradient, and to quantify the relative importance of benthic and pelagic compartments.

Temporal variability in open-water metabolic rates

Our modelled ecosystem metabolism from continuous oxygen measurements showed large variations in GPP, R and NEP on a daily basis and across seasons and habitats. The magnitude and temporal variability in open-water metabolic rates resembled those found for North American estuaries (Caffrey 2004). Similar to Caffrey (2004), we found that the balance between GPP and R was largely determined by the habitat type. Accordingly, our shallow sites inside or just outside the eelgrass meadows were significantly more net autotrophic as compared to the more net heterotrophic, deeper muddy site with greater dominance of pelagic processes. As observed in other studies in shallow estuaries and lakes, our daily NEP estimates indicated large shifts from net autotrophy to net heterotrophy over both seasonal (monthly) and even daily time scales (eg. Staehr & Sand-Jensen 2007, Kemp & Testa 2011). At the central deeper station, there was a general pattern of spring net autotrophy transitioning to summer heterotrophy, consistent with a long-term analysis of net oxygen exchanges in Roskilde Fjord (Staehr et al. 2017). In comparison, the shallow sites showed less seasonality in NEP remaining mostly positive throughout the year, although with a clear drop in productivity during late autumn when light and temperature decreased and storm-related loss of vegetation occurred. Our residual analysis showed that day-to-day variability was clearly larger at the central pelagic-dominated station for both GPP and R

426   and to a lesser extent for NEP. This suggests that deeper stations dominated by pelagic processes

427   and with stronger light limitation are more sensitive to changes in nutrient conditions and incoming

428   light.

429

430   *Ecosystem integrated metabolic rates*

431   Across the three investigated habitats, summer (June through August) mean daily rates of GPP in

432   Roskilde Fjord (7.0 ± 0.3 mg O_2 m^{-2} d^{-1}; Mean $\pm$ SE) are slightly lower than those reported by

433   Hoellein et al. (2013) for a large range of estuaries across the Northern hemisphere (~10 ± 1 g O_2

434   m^{-2} d^{-1}). Interestingly respiration rates in Roskilde Fjord are less than half (6.0 ± 0.3 g O_2 m^{-2} d^{-1})

435   than those reported by Hoellein et al. (2013): (~14 ± 1 g O_2 m^{-2} d^{-1}). Thus, where many estuaries

436   display strong net heterotrophy, even during summer, Roskilde Fjord is overall net autotrophic

437   (1.0 ± 0.3 g O_2 m^{-2} d^{-1}) in agreement with a small subset of shallow densely vegetated estuaries

438   around the USA (Caffrey 2004) and Chesapeake Bay tributaries (Kemp & Testa 2011). Our results

439   are similar to those of Hume et al. (2011) who applied the eddy correlation technique to measure

440   productivity during summer (June and July), reporting NEP in vegetated shallow areas of 0.6 g O_2

441   m^{-2} d^{-1}.

442

443   Using a molar $O_2 : C$ quotient of 1, and using a simple polynomial model (Laas et al. 2012) to

444   extend the measured GPP period (April to December) to cover the entire year, Roskilde Fjord had

445   an annual GPP ranging between 575 and 663 g C m^{-2} y^{-1} across the muddy and eelgrass habitats.

446   These values are in the high end of the spectrum compared to GPP values reported by Borum &

447   Sand-Jensen (1996) for 34 coastal systems (median 346; range 28 to 820 g C m^{-2} y^{-1}) and the

448   nearby Limfjorden where GPP was estimated to have reached 350 g C m^{-2} y^{-1} during pristine

449   conditions (Krause-Jensen et al. 2012). Annual NEP ranged from -35 g C m^{-2} y^{-1} in the muddy

450   area to 74 g C m^{-2} y^{-1} in the vegetated area, similar to previous findings by Caffrey (2004) using

451   the open-water oxygen technique (range -55 to 68 g C m^{-2} y^{-1}) in five shallow estuaries dominated

452   by submerged aquatic vegetation. In comparison, net primary production, estimated from net

453   accumulation of plant biomass, may be several times higher within dense beds of perennial

454   macrophytes (Borum & Sand-Jensen 1996). For example, Wium-Andersen & Borum (1984)

455   estimated annual (April to October) net above-ground production of 814 g C m^{-2} y^{-1} in a Danish

456   estuary. The significantly lower NEP rates obtained from the open-water oxygen measurements

457   are not surprising as this technique accounts for oxygen production and consumption by both auto-

458   and heterotrophs occupying both benthic and pelagic components (Staehr et al. 2012b). In addition,

459   depending on water exchange, the oxygen technique will integrate processes that can easily exceed

460   the area of the densely vegetated macrophyte beds (Hume et al. 2011). In support of this, NEP

461   rates measured within benthic chambers under calm wind conditions (2 to 4 m s^{-1}) in June were up

462   to 4 times higher than NEP rates calculated from parallel open-water oxygen measurements within

463   the dense eelgrass bed.

464

465   *Importance of benthic and pelagic contributions*

466   To distinguish benthic and pelagic contributions to total areal rates of GPP, R and NEP, we applied

467   24-hour oxygen chamber incubations and compared these with daily rates calculated from open-

468   water oxygen measurements. The analytical approach was essentially the same for benthic, pelagic

469   and open-water measurements, with the modification that incubations did not account for air-water

470   gas exchange.

471

472 Pelagic rates of metabolism measured in this study were similar to rates reported by Mantikci

473 (2014) for the central part of Roskilde Fjord, where a combination of ^{14}C and oxygen incubation

474 techniques was used. Converting rates by Mantikci (2014) using a molar O$_2$: C quotient of 1, GPP

475 ranged between 0.3 and 5.3 mg O$_2$ m^{-2} d^{-1}; R between 0.3 and 3.2 mg O$_2$ m^{-2} d^{-1} and NPP between

476 0.1 and 3.5 mg O$_2$ m^{-2} d^{-1}. These levels are consistent with those reported here (Figure 6) and both

477 studies report similar seasonality with peaks in June and July. Oxygen fluxes across the water-

478 sediment interface were measured in light and dark incubations of sediment cores as part of the

479 DNAMAP, including St. 60 in Roskilde Fjord (Dalsgaard 2003). Dalsgaard (2003) reported

480 seasonal ranges in GPP from 0.1 to 1.5 g O$_2$ m^{-2} d^{-1}; respiration from 0.3 to 2.0 g O$_2$ m^{-2} d^{-1}, and

481 NEP from -0.7 to 0.5 g O$_2$ m^{-2} d^{-1}. These levels resemble those measured for the muddy station

482 (Table 4) with a monthly variation mirroring that of water temperature (Figure 6). Thus, pelagic

483 and benthic rates reported in this study have similar levels and temporal variability as those

484 previously reported.

485

486 Comparing open-water (total) rates with pelagic and benthic incubations, demonstrated that

487 benthic metabolism is important and often dominating in shallow coastal environments. This was

488 particularly prominent in the eelgrass area where the benthic habitat alone accounted for

489 approximately 90% of total GPP and R and 64% of NEP. While the unvegetated shallow site had

490 total metabolic rates very similar to the nearby eelgrass site, the sum of the measured benthic and

491 pelagic rates could only account for 37% of the open-water GPP estimates and 54% of the

492 ecosystem respiration rates (Fig. 5). An even greater discrepancy between total and the sum of

493 pelagic and benthic rates was found for the muddy site. We attribute this difference to the

494 metabolic footprint of the eelgrass meadow markedly exceeding beyond the confines of the

495 meadow, thereby supplying oxygen to the neighboring unvegetated site as well as the deeper

496 central sites. We interpret the observed systematic differences in metabolism between areas

497 differing substantially in substrate conditions and vegetation cover, mainly as differences between

498 different habitats, rather than just spatial differences related to factors such as differences in water

499 depth. This terminology agrees with Caffrey (2004) who also categorized her sites based on the

500 dominant habitat adjacent to the deployment site.

501

502 The importance of benthic processes was not surprising given the shallowness of the estuary and

503 its relatively clear waters, providing sufficient light at the sediment surface supporting benthic

504 primary production throughout the studied areas. Previous evaluations of the benthic vs. pelagic

505 contribution to the inner part of Roskilde Fjord suggested that phytoplankton contributed between

506 100 and 62% of total areal GPP (Borum & Sand-Jensen 1996). However, water quality has

507 improved significantly since the 1990s, especially in the inner parts of the estuary (Staehr et al.

508 2017). Accordingly, Chl a has decreased by 50% and Secchi depth has increased by approximately

509 1 m enabling eelgrass to expand from 2 to 2.6 m depth, and the improved light environment has

510 likely stimulated the growth of benthic algal communities (Sand-Jensen & Borum 1991, Staehr et

511 al. 2017). It is therefore plausible that the benthic compartment now plays a much larger role

512 compared to the 1990s. This change should render the system more autotrophic and a greater sink

513 for carbon and nutrients (McGlathery et al. 2007, Fourqurean et al. 2012) stored in the sediments

514 by accumulation of slowly degradable roots and rhizomes and allochtonous organic matter trapped

515 by the seagrasses (Kennedy et al. 2010, Duarte et al. 2013).

516

517

518 *Depth gradients in metabolic rates*

519 Shallow areas dominated by benthic vegetation maintaining large standing carbon stocks tend to

520 have a higher NEP compared to deeper waters dominated by phytoplankton of lower standing

521 stocks (e.g., Nixon et al. 2001, Caffrey et al. 1998, Caffrey 2004). In agreement with this, we

522 observed a clear trend with decreasing GPP, R and NEP from the shallow sites toward the central

523 deeper habitat. Differences in metabolic rates among the three investigated areas were much more

524 pronounced when comparing volumetric rates (rather than areal rates), which for GPP were 2.5

525 fold lower at the muddy deeper site compared to the two shallow sites, thus reflecting that the same

526 area-specific production was concentrated in a smaller volume at the shallow sites with benthic

527 dominance. Similar conclusions were made by Odum in 1967 who found a clear declining trend

528 in oxygen concentration with increasing depth, reflecting higher metabolic rates (on a volumetric

529 basis) in shallower waters, where temperatures are higher and plant pigments are more

530 concentrated (Kemp & Testa 2011). It is a general feature that the compression of the photic zone

531 from several meters in phytoplankton communities to centimeters-meters in macrophyte stands to

532 mm scale in dense microalgal mats is paralleled by a marked increase in volumetric photosynthesis

533 while areal photosynthesis remains remarkably constant (Krause-Jensen & Sand-Jensen 1998).

534 The observed shift from positive towards negative NEP with increasing depth and distance from

535 the shore also supports the concept that non-advective physical processes drive a net transport of

536 organic matter from shallow to deeper areas (Kemp et al. 1997, Van de Bogert et al. 2007).

537 While gradients in metabolic rates were observed with increasing depth and distance from the

538 dense eelgrass meadows, habitat differences were less obvious in the data set covering a temporal

539 range from April to December. As an example, the shallow eelgrass and bare (~unvegetated) areas

540 had almost identical magnitude and seasonality in GPP, R and NEP, despite the much lower

24

541   benthic rates measured here. The discrepancy between total rates and the sum of benthic and

542   pelagic rates in the bare habitat, suggests that open-water rates measured in this habitat were

543   strongly affected by excess oxygen produced in the nearby dense eelgrass meadow. Although the

544   muddy central site had comparable higher pelagic contribution, there was also a discrepancy

545   between total rates and incubations here, suggesting that the oxygen spillover effect from dense

546   eelgrass beds can be an important feature affecting oxygen dynamics in an area that far exceeds

547   the local meadow.

548

549   *Importance of environmental conditions for temporal variability in metabolic rates*

550   Despite the observed differences in day-to-day variability of metabolic rates among the shallow

551   and deeper areas, they displayed similar temporal variation and magnitude in open-water metabolic

552   rates, having quite similar responses to abiotic conditions in terms of increasing net autotrophy

553   during calm, warm and sunny periods. Similar temporal variation and relationships with

554   meteorological drivers characterize productive temperate lake ecosystems (Laas et al. 2012, Staehr

555   et al. 2007, 2010). Other studies of estuarine ecosystem metabolism have, however, reported weak

556   relationships between NEP and prevailing temperature (negative), light (positive) and wind

557   (negative) conditions (Caffrey 2004, Hoellein et al. 2013, Murrell et al. 2018). Studies of GPP, R

558   and NEP using the eddy correlation technique, have reported substantial day-to-day variability

559   strongly coupled to variations in light and hydrodynamic conditions (Hume et al. 2011, Lee et al.

560   2017). Based on the eddy correlation technique, that takes into account the lateral exchange of

561   oxygen, significant differences in metabolic rates have also been identified between a seagrass site

562   and a nearby unvegetated site (Hume et al. 2011). This suggests that the parameterization of diel

563   oxygen changes applied in our study is not sufficiently sensitive to separate such lateral exchanges

564   between neighboring sites. Interestingly, a recent study has shown how the difference between

565   open water and bottle incubations of DO can be used as a proxy of benthic contribution, which in

566   shallow seagrass-dominated environments can be important, indicating that the open-water

567   method can capture both water column and benthic processes (Murrell et al. 2018).

568

569   Inability of the open-water oxygen technique to account for lateral oxygen exchanges in

570   calculations of metabolic rates likely explains the apparently strong effects of wind observed at

571   the shallow sites (Fig. 8D and 9). The larger diel oxygen excursions at the shallow (2 to 3 m) sites

572   were almost instantly reduced to levels and variations corresponding to those of at the deeper (4

573   to 5 m) sites when wind conditions (wind speed and direction) forced a significant (~20 to 30 cm)

574   shift in water levels. Larger uncertainties in calculated metabolic rates are therefore likely to occur

575   on cold, windy and cloudy days where diel changes in oxygen are mostly governed by physical

576   forcing rather than by biological activity, which our model is insufficient to capture. We, therefore

577   excluded those extreme days with poor model performance ($r^2 < 0.2$), which typically represented

578   situations when water levels increased and surface irradiance was low, observed as drops in

579   calculated GPP (and NEP; not shown). Still after excluding days with poor model fit, there was a

580   strong negative effect of wind speed on apparent GPP and NEP (Fig. 8). The choice of models to

581   correct for the effect of wind on the exchange of oxygen across the air-water interface can affect

582   calculated metabolic rates (Marino & Howarth 1993). Sensitivity studies, however, show that this

583   issue is a greater problem in oligotrophic systems, with small diel variations in oxygen

584   concentrations around saturation (Staehr et al. 2010),which was seldom the case in the shallow

585   eelgrass-dominated habitat. Finally, as air-sea exchange is more effective in the surface layers

586   (Wannikhof et al. 2009), wind-induced turbulence has a proportionately larger effect on the

587 metabolic rate calculations in shallow than in deep water (Caffrey 2004). Other studies have

588 explained the negative effect of wind on metabolic rates as a result of lower light availability,

589 either due to deeper mixing of phytoplankton (e.g. Staehr & Sand-Jensen 2007, Hu et al. 2015),

590 elevated levels of suspended particles (e.g. Arfi et al. 1993) or co-variation with wind and reduced

591 surface light from cloud cover (Fisher et al. 2003). Elevated respiration caused by wind-induced

592 resuspension of labile organic matter from the sediment surface into the water column (Demers et

593 al. 1987) may also reduce NEP (Dokulil 1994). Nevertheless, our results indicate that a significant

594 dilution or spillover effect occurs between adjacent habitats. Except for periods with very limited

595 water movement, it therefore seems appropriate to think of the measured open-water metabolic

596 rates as apparent, rather than absolute rates of local production and respiration. While this is a

597 weakness of the open-water oxygen method for determining site-specific metabolic rates, it

598 supports the use of this technique when aiming to evaluate system-integrated rates across larger

599 heterogeneous areas as compared to compartment specific rates where incubation techniques are

600 preferable (Kemp and Testa 2011, Staehr et al. 2012b).

601

602 *Conclusions*

603 This study confirms the important functional and biogeochemical role of eelgrass meadows in the

604 coastal systems. Besides their local importance as biologically diverse and productive hot spots, a

605 considerable spillover of oxygen from seagrass meadows to neighboring areas imply an ecological

606 and biogeochemical impact extending beyond their physical coverage. Furthermore, this spillover

607 effect is not limited to oxygen, but concerns other biogeochemically relevant substances as well,

608 such as organic carbon and nutrients. Our study suggests that the extent of this footprint is strongly

609 affected by prevailing wind forcing (Fig. 10), but surface irradiance, local physical characteristics

610 (depth, fetch), sediment characteristics (sediment grain size, organic matter content), density of

611 aquatic vegetation and water quality (Secchi depth and Chl *a* concentration) also modulate this

612 effect. While other methods, such as the eddy correlation technique, are superior in accounting for

613 lateral exchanges, our study shows that open-water oxygen measurements in combination with

614 chamber incubations are well suited to assess the importance of different habitats and

615 environmental conditions for spatio-temporal variability in estuarine metabolism.

616

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Tables

Table 1. Overview of sampling program and subsequent modeling. Study sites are shown in Figure 1.

Study component	Study sites	Study period
Water quality sampling	Mud	May-Dec 2015
Metabolic rates - seasonal study		
- Diel O$_2$ measurements (open)	Mud, Eelgrass, Bare	Apr-Dec 2015
- Benthic incubations	Eelgrass, Bare	3 occasions: Apr, Jun, Aug 2015
- Pelagic incubations	Mud	14 occasions: Apr-Dec 2015
Metabolic rates - short term study		
- Diel O$_2$ measurements (open)	Depth gradient across habitat types	26. Apr-20. May 2016
Modeling of metabolic rates	Mud, Eelgrass, Bare	Jan-Dec

818   Table 2. GLMM model results for effects of habitats and seasons on differences in total (open

819   water), pelagic and benthic (chamber) rates of gross primary production (GPP), respiration (R)

820   and net ecosystem production (NEP). Significant levels (p<0.05) effects are highlighted in bold.

821   Degrees of freedom (DF) for the numerator (Num) and the denumerator (Den) along with the F-

822   values are shown.

Type	Variable	Effect	Num DF	Den DF	F-value	p
Total	GPP	**habitat**	**2**	**387**	**7.4**	**<0.001**
		month	**7**	**219**	**25.6**	**<0.001**
		habitat*month	14	387	1.1	0.352
	R	**habitat**	**2**	**381**	**4.5**	**0.011**
		month	**7**	**219**	**7.3**	**<0.001**
		habitat*month	14	381	1.2	0.244
	NEP	**habitat**	**2**	**381**	**4.4**	**0.013**
		month	**7**	**219**	**3.6**	**<0.001**
		habitat*month	14	381	1.4	0.155
Pelagic	GPP	month	6	7	2.0	0.187
	R	month	6	7	1.1	0.463
	NEP	month	6	7	1.3	0.369
Benthic	GPP	**month**	**2**	**14**	**30.1**	**<0.001**
		Habitat	**2**	**14**	**47.6**	**<0.001**
		month*Habitat	**3**	**14**	**13.7**	**<0.001**
	R	**month**	**2**	**14**	**12.8**	**0.001**
		Habitat	**2**	**14**	**15.3**	**<0.001**
		month*Habitat	**3**	**14**	**2.9**	**0.072**
	NEP	**month**	**2**	**14**	**13.2**	**0.001**
		Habitat	**2**	**14**	**4.0**	**0.043**
		month*Habitat	**3**	**14**	**4.2**	**0.026**

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824   Table 3. Correlation table between daily values of water temperature (Tw), surface irradiance

825   (PAR), wind speed at 10 m (Wind), gross primary production (GPP), ecosystem respiration (R)

826   and net ecosystem production (NEP) at the shallow eelgrass site (Eelgrass) and non-eelgrass site

827   (Bare) and the central deeper St60 (Muddy) habitat. The Spearman rank correlation coefficient

828   (r_s) is shown with the significance levels (p).

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Habitat	Parameter	GPP		R		NEP	
		r_s	p	r_s	p	r_s	p
Eelgrass	Tw	0.61	<0.0001	0.28	<0.0001	0.32	<0.0001
	PAR	0.53	<0.0001	0.15	0.0235	0.34	<0.0001
	Wind	-0.08	0.2491	0.15	0.0224	-0.18	0.0067
Bare	Tw	0.65	<0.0001	0.26	<0.0001	0.30	<0.0001
	PAR	0.52	<0.0001	0.09	0.1775	0.36	<0.0001
	Wind	-0.10	0.1362	0.29	<0.0001	-0.36	<0.0001
Mud	Tw	0.46	<0.0001	0.39	<0.0001	0.17	0.0187
	PAR	0.54	<0.0001	0.20	0.006	0.48	<0.0001
	Wind	-0.08	0.3125	0.25	0.0006	-0.36	<0.0001

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838   Table 4. *In-situ* determined benthic rates (g O_2 m^{-2} d^{-1}) of gross primary production (GPP),

839   respiration (R) and net production (NEP) for three different habitats (Eelgrass, Bare sediment

840   and Muddy sediment) in Roskilde Fjord. Values are means of triplicates ± SD.

Habitat	Process	April	June	August
Eelgrass	GPP	0.4 ± 0.5	8.8 ± 2.6	3.5 ± 1.8
	NEP	0.2 ± 0.4	2.1 ± 1.5	-1.6 ± 4.2
	R	-0.2 ± 0.1	-6.7 ± 2.2	-5.1 ± 5.5
Bare	GPP	0.3 ± 0.9	1.7 ± 0.8	1.7 ± 3.7
	NEP	-0.2 ± 0.4	-0.4 ± 0.7	-2.4 ± 2.6
	R	-0.5 ± 0.6	-2.1 ± 1.4	-4.0 ± 5.0
Muddy	GPP	Nd	1.0 ± 2.4	0.3 ± 0.7
	NEP	Nd	-1.0 ± 1.6	-1.4 ± 1.4
	R	Nd	-2.0 ± 1.4	-1.8 ± 2.1

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Figure legends

843 Figure 1. Location of the study sites in Roskilde Fjord, Denmark. Investigations were performed

844 in two areas of the inner fjord with St60 representing the deeper (5 m) muddy (Mud) area, and

845 the shallower (2-3 m) experimental area (Exp) representing a vegetated (Eelgrass) and a non-

846 vegetated (Bare) site. The color scale in the central figure is a model estimate of the cover (0 to

847 100%) of eelgrass plants (Mohn et al. 2015).

848 Figure 2. Techniques applied to measure metabolic rates in different habitats: A) *in-situ* chamber

849 for benthic oxygen fluxes, B) bottle incubations for pelagic primary production and respiration

850 rates, C) buoy with oxygen sensor at the open-water muddy site (St60).

851 Figure 3. Seasonal changes in A) daily water temperature at 1 and 4 m depth and surface

852 irradiance (PAR), B) daily wind speed 10 m above sea level, C) Secchi depth and Chl *a*

853 (biweekly) and D) dissolved inorganic phosphorus (DIP) and nitrogen (DIN) (biweekly).

854 Figure 4. Daily estimates of gross primary production (GPP), ecosystem respiration (R) and net

855 ecosystem production (NEP) determined from continuous oxygen measurements in two shallow

856 (2-3 m) sites dominated by A) eelgrass and B) bare sediments, and C) in the deeper (5m) muddy

857 site in Roskilde Fjord, Denmark (April-December 2015). R is presented as a negative number to

858 facilitate plotting on the same graph as GPP and NEP. Negative NEP values are days when R

859 outpaces GPP. Solid lines are 14-d moving averages. Mean GPP, R and NEP for the three sites

860 over the study period are summarized in D) with 95% confidence intervals.

861 Figure 5. Comparison of GPP, R and NEP mean rates for the three studied habitats in Roskilde

862 Fjord obtained from open-water oxygen measurements, representing total rates, and bottle

863 incubations representing pelagic rates and benthic chamber rates. Means represent April-

864   November for total rates, April-September and December for pelagic rates, and April, June and

865   August for benthic rates (cf. Fig. 5). Error bars show 95% confidence intervals of the means.

866   Figure 6. Monthly means of pelagic, benthic and open-water (total) rates of GPP, R and NEP in

867   Roskilde Fjord estimated from the GLMM. Means represent an average of the three habitats and

868   error bars show the 95% confidence interval of the mean values. Benthic rates marked with

869   hatched bars were calculated as the difference between total and pelagic rates.

870   Figure 7. Differences in GPP, R and NEP rates across a shallow depth gradient in Roskilde

871   Fjord. Rates are means over 24 days with 95% confidence interval.

872   Figure 8. Time series of A) wind speed and direction, B) water level and surface light, C) oxygen

873   saturation level and D) GPP during a four week spring period in Roskilde Fjord. In C and D the

874   shallow (1.8 to 2.1 m) and deeper part (4.1 to 5 m) of the estuary are contrasted, using data from

875   the transect investigation. Shaded areas highlight periods with changes in water level related to

876   changing wind speed and direction.

877   Figure 9. Relationship of wind speed with daily rates of A) GPP and B) NEP at shallow (1.8 to

878   2.1 m) and deeper sites (4.1 to 5 m).

879   Figure 10. Conceptual diagram for depth gradients in primary production and respiration from

880   shallow nearshore to deeper central parts of Roskilde Fjord, under calm (left) and windy

881   conditions (right). At shallower depths, light conditions favor benthic primary producers such as

882   eelgrass in soft sandy habitats. These plants have higher areal GPP and a net positive oxygen

883   balance. With increasing depth, planktonic and benthic microalgae capable of utilizing the high

884   ambient nutrient levels and relatively high light levels dominate GPP and R. Muddy sediments at

885   deeper depths prevent any significant establishment of macrophytes and contribute with high

886   oxygen demand, resulting in negative NEP. Increasing wind efficiently mixes water from deeper

887   areas onto shallow nearshore habitats, containing lower oxygen concentrations and higher levels

888   of suspended matter, thereby reducing water transparency and elevating pelagic oxygen
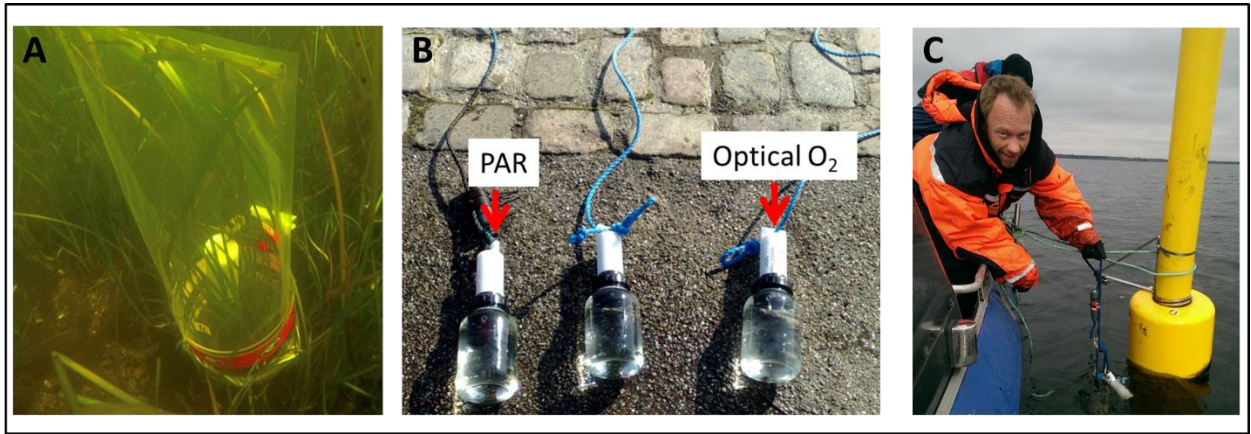
889   consumption.

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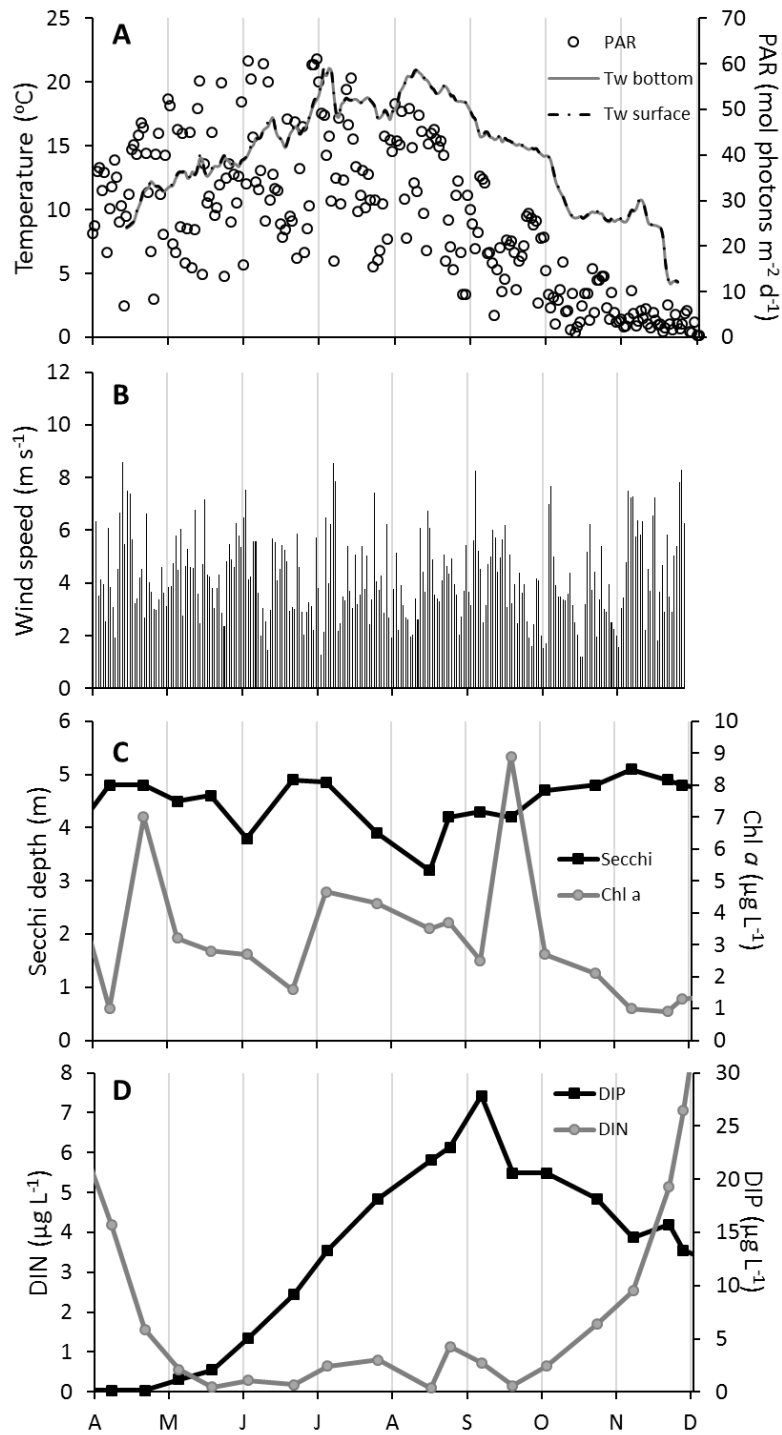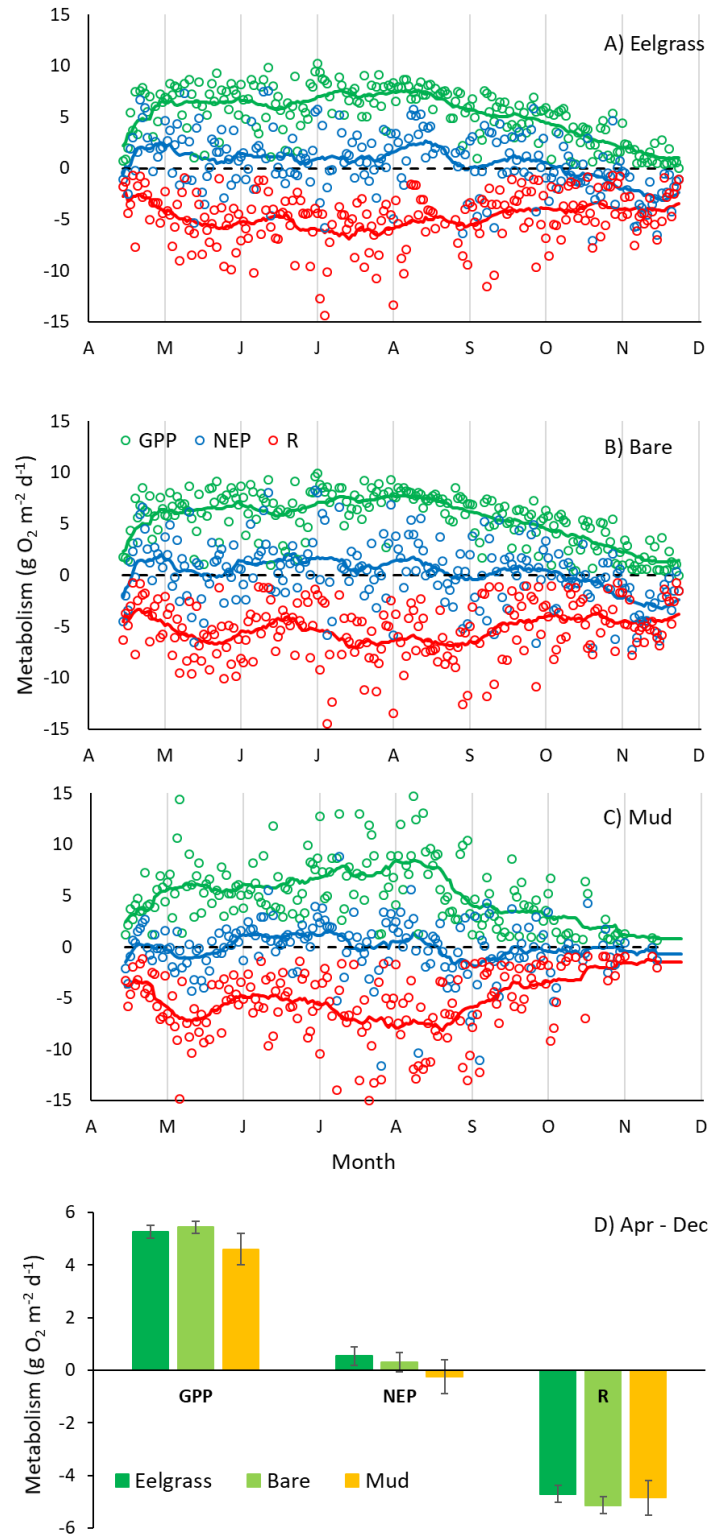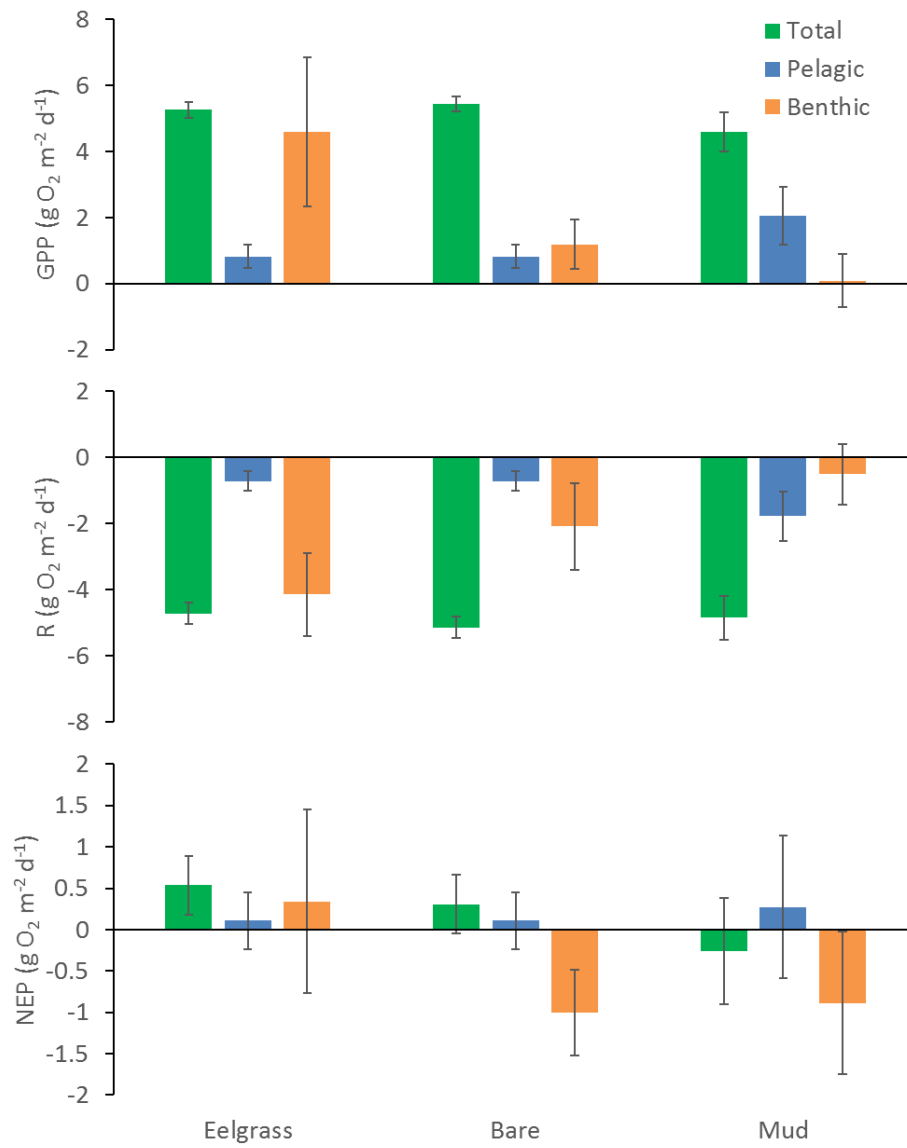891   Figures

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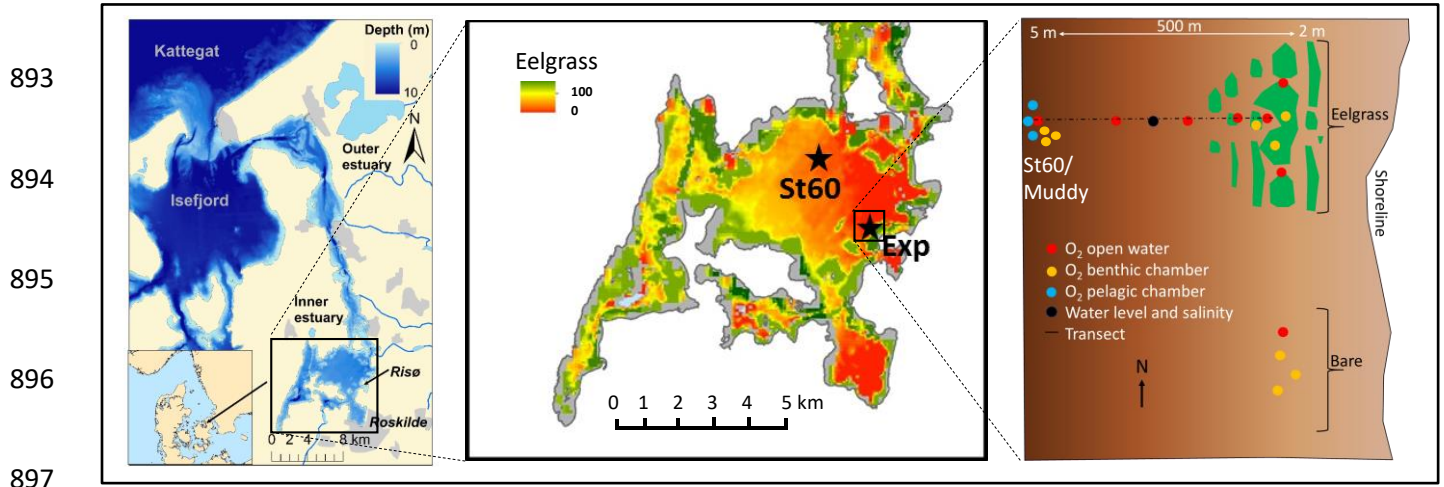
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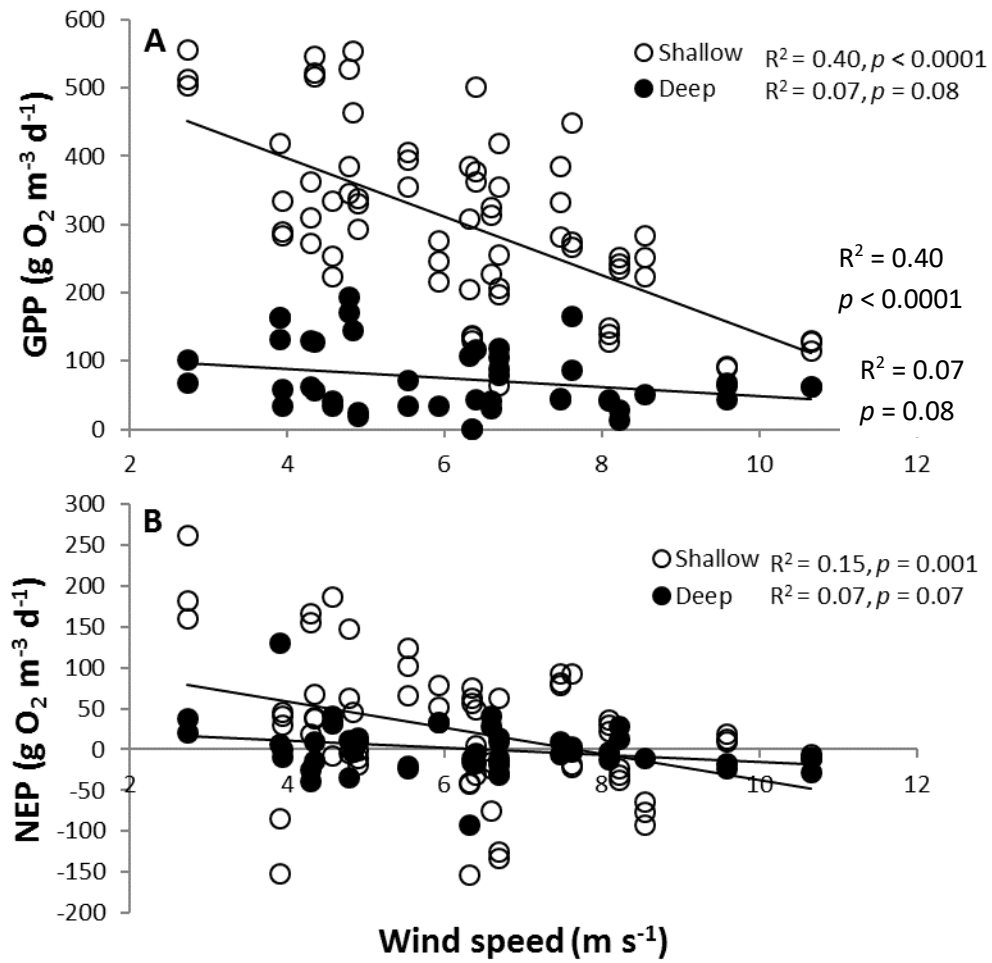
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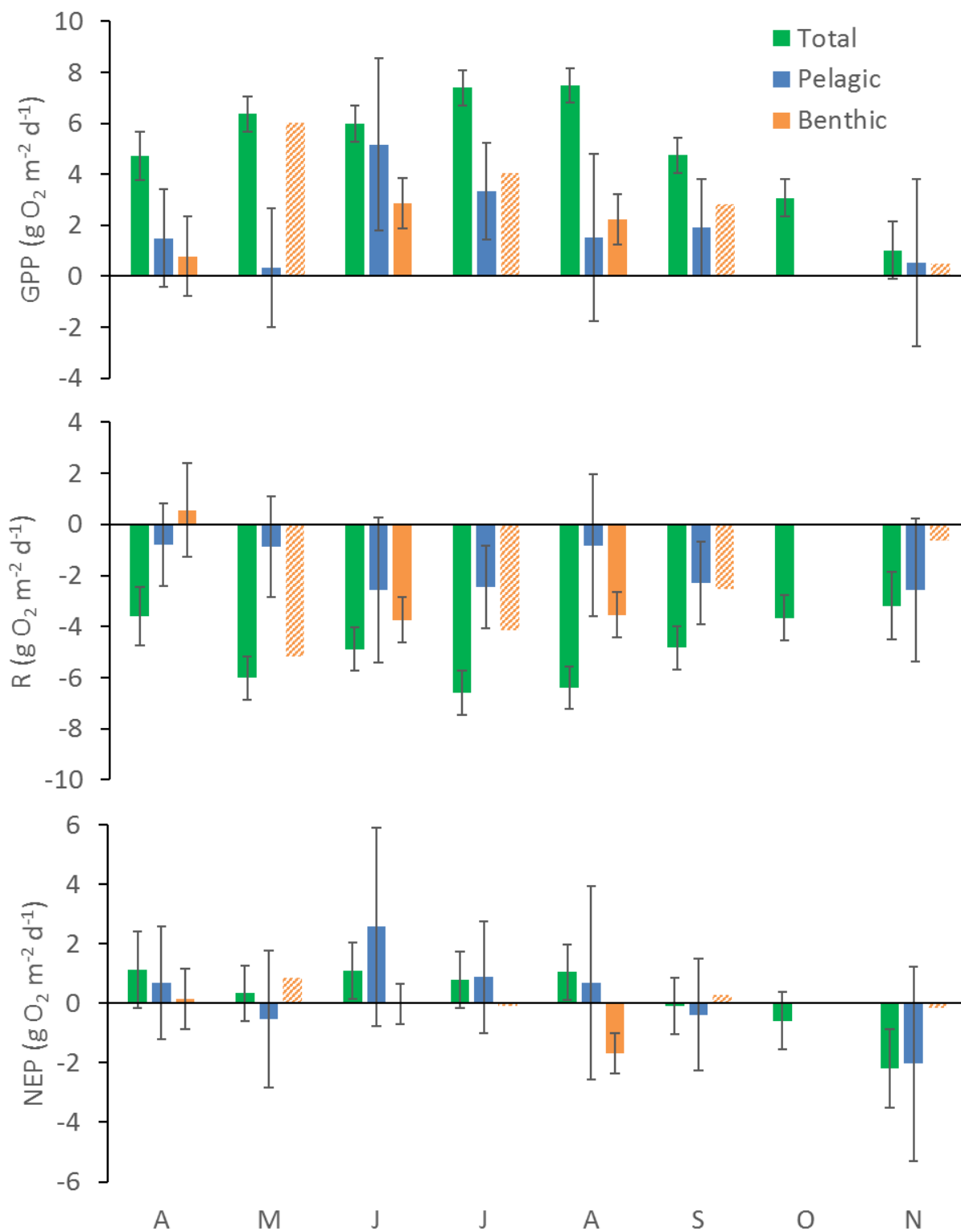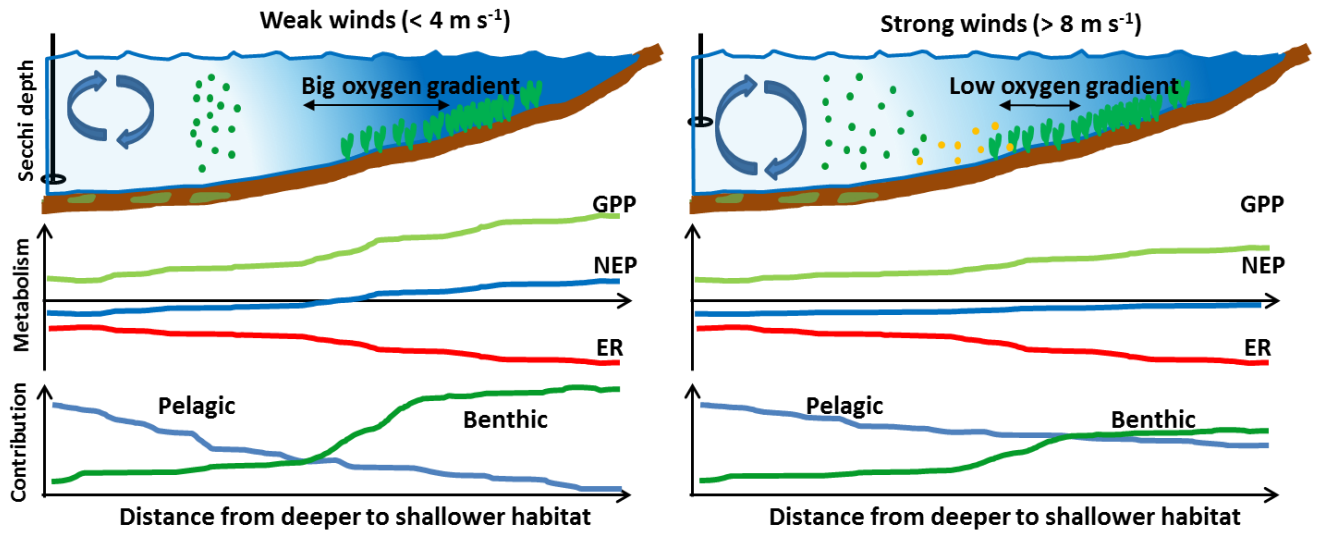
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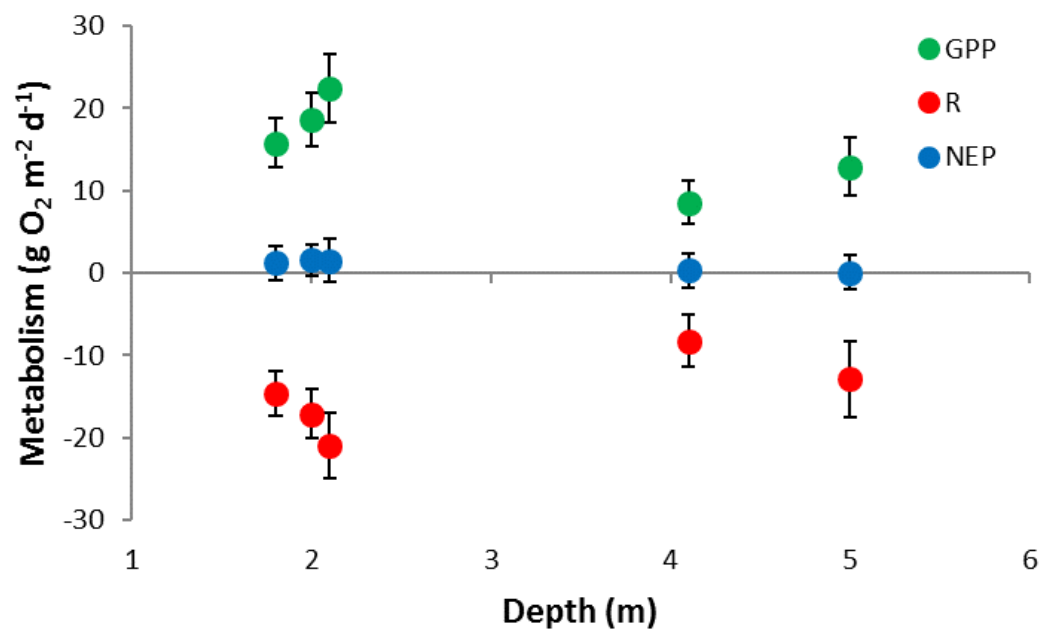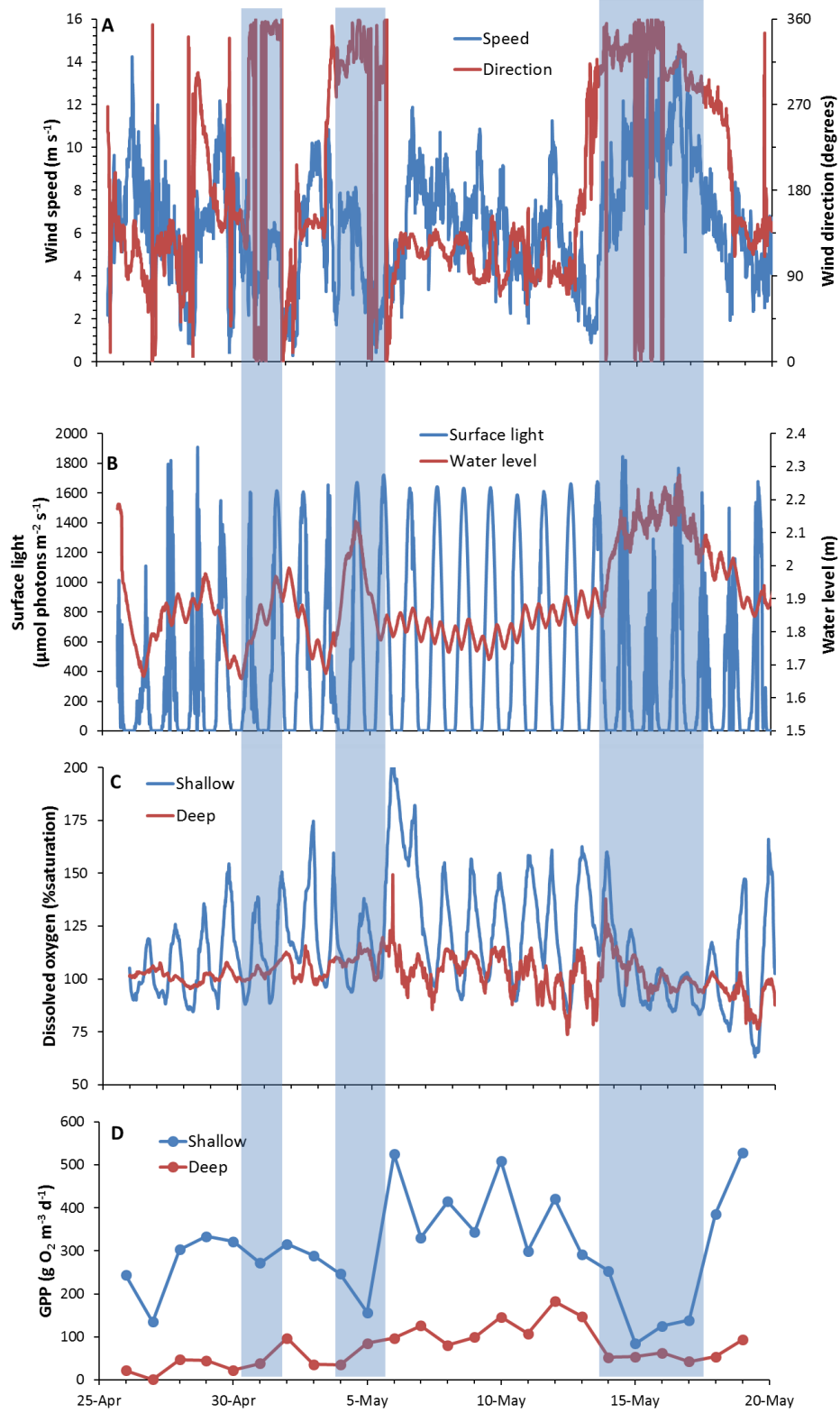
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