

Title: Abiotic and biotic interactions in the diffusive boundary layer of kelp blades create a potential refuge from ocean acidification

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Short title: Living in the DBL of kelp blades

1 Summary

- 2 1. Seaweeds are able to modify the chemical environment at their surface, in a micro-zone called
3 the diffusive boundary layer (DBL), via their metabolic processes controlled by light intensity.
4 Depending the thickness of the DBL, sessile invertebrates such as calcifying bryozoans or
5 tube-forming polychaetes living on the surface of the blades can be affected by the chemical
6 variations occurring in this micro-layer. Especially in the context of ocean acidification, these
7 microhabitats might be considered as a refuge from lower pH, because during the day
8 photosynthesis temporarily raises the pH to values higher than in the mainstream seawater.
- 9 2. We assessed the thickness and the characteristics of the DBL at two pH levels (today's average
10 surface ocean pH 8.1 and a reduced pH predicted for the end of the century, pH 7.7) and
11 seawater flows (slow, 0.5 and fast, $> 8 \text{ cm s}^{-1}$) on *Ecklonia radiata* (kelp) blades. Oxygen and
12 pH profiles from the blade surface to the mainstream seawater were measured with O_2 and pH
13 microsensors for both bare blades and blades colonized by the bryozoan *Membranipora*
14 *membranacea*.
- 15 3. The DBL was thicker in slow compared to fast flow and the presence of bryozoans increased
16 the DBL thickness and shaped the DBL gradient in dark conditions. Net production was
17 increased in the low pH condition, increasing the amount of oxygen in the DBL in both bare
18 and epiphytized blades. This increase drove the daily pH fluctuations at the blade surface,
19 shifting them towards higher values compared to today's pH. The presence of bryozoans led
20 to lower oxygen concentrations in the DBL and more complex pH fluctuations at the blade
21 surface, particularly at pH 7.7.
- 22 4. Overall this study, based on microprofiles, shows that, in slow flow, DBL micro-environments
23 at the surface of the kelps may constitute a refuge from ocean acidification with pH values
24 higher than those of the mainstream seawater. For calcifying organisms, it could also represent
25 training ground for harsh conditions, with broad daily pH and oxygen fluctuations. These

26 chemical micro-environments, biologically shaped by the macrophytes, are of great interest
27 for the resilience of coastal ecosystems in the context of global change.

28

29 **Key-words (5):** bryozoan, ecomechanics, epiphytism, hydrodynamic, seaweed, microhabitat,
30 pH

31

32 **Introduction**

33 Seaweeds are not only the dominant primary producers in coastal waters but they are
34 important bioengineers that are able to modify their surrounding environment (Hurd *et al.*
35 2014). In particular, brown macroalgae forming broad communities such as the Laminariales
36 and Fucales act as ecosystem engineers by influencing physical factors including seawater
37 velocity (Gaylord *et al.* 2007; Rosman *et al.* 2010) and light penetration (Reed & Foster 1984),
38 and the chemical characteristics of the mainstream seawater, including carbonate chemistry
39 (Delille, Borges & Delille 2009; Cornwall *et al.* 2013a; Hendriks *et al.* 2014), oxygen and
40 nutrient availability (Frieder *et al.* 2012; Saderne *et al.* 2015). Seaweed communities can
41 modulate their surrounding chemical environment on seasonal and diel cycles (Delille *et al.*
42 2000; Saderne, Fietzek & Herman 2013), however the most rapid and variable fluctuations in
43 chemical parameters occur in the microenvironments formed at the surface of the seaweeds, in
44 a zone called the diffusive boundary layer (DBL, also termed the concentration boundary layer)
45 (Hurd *et al.* 2014; Wahl, Saderne & Sawall 2016).

46 The DBL is a discrete micro-layer at the surface of many organisms (e.g. Kühl *et al.*
47 1995; de Beer *et al.* 2000), including all primary producers (e.g. Koch 1994; Brodersen *et al.*
48 2015), that buffers them from the mainstream seawater (Vogel 1999). This specific layer is
49 formed when a fluid flows over a solid, such as a kelp blade. The no-slip condition creates a

50 region of viscously-dominated laminar flow at the seaweed surface. In this thin laminar layer,
51 movement of ions and molecules is by molecular diffusion, and the metabolic activity of the
52 organism results in a concentration gradient due to the uptake and release of dissolved
53 substances to and from the organism's surface (Vogel 1999; Hurd 2000). Fluctuations observed
54 in these microhabitats at the surface of seaweed are mainly driven by their photosynthesis and
55 respiration processes under the control of light (Sand-Jensen, Revsbech & Jørgensen 1985;
56 Hurd *et al.* 2011; Cornwall *et al.* 2013b ; Hofmann, Koch & de Beer 2016). Thus, metabolic
57 activity affects the micro-chemical environment of the DBL which differs from that in the
58 mainstream seawater just micrometres away (Hurd 2015), with implications for the alga itself
59 and all the other small organisms living on the blades.

60 The importance of DBLs in controlling the availability and transfer of nutrients and
61 metabolites to and from algal surfaces is well known (Raven 1997; Hurd 2000; De Beer &
62 Larkum 2001). The presence of a thick DBL formed under slow flows ($< 2 \text{ cm sec}^{-1}$) may, for
63 example, decrease the uptake of dissolved inorganic carbon and nutrients (Wheeler 1980;
64 Kregting *et al.* 2011). However, these microenvironments are beneficial for other processes
65 such as timing of gamete release (Pearson, Serrão & Brawley 1998) or keeping antifouling
66 agents at their surface (Dworjany, De Nys & Steinberg 2006). Interestingly, different
67 organisms such as bacteria, diatoms, larvae and spores live in this thin layer (Schaffelke 1999;
68 Wahl, Saderne & Sawall 2016). Some bigger and calcifying species such as bryozoans or tube
69 forming worms also settle on brown macroalgal blades (Saderne & Wahl 2013) and would be
70 submitted to the fluctuations occurring in these micro-habitats. In particular, bryozoans are
71 known to be epiphytic organisms which influence their algal substratum (Muñoz, Cancino &
72 Molina 1991; Hurd *et al.* 1994a), changing for example nutrient uptake at the seaweed surface
73 (Hurd *et al.* 1994a; Hepburn & Hurd 2005; Hepburn, Frew & Hurd 2012). According to their
74 size, their structure and their direct contact with the algal substratum, they are typically sessile

75 organisms which would be affected by oxygen and pH fluctuations that occur in the DBL (Irwin
76 & Davenport 2002; Wahl, Saderne & Sawall 2016). Nevertheless, there is a knowledge gap on
77 the interactive effects that bryozoans can have with other abiotic factors of the surrounding
78 water and their role in the formation and gradient creation in the DBL has never been clearly
79 observed.

80 The different physical and chemical parameters of the environment surrounding
81 macroalgae can affect the thickness of the DBL and the flux of dissolved substances, and thus
82 the concentration gradient which can vary from μm to mm (0.1 to 10 mm; Raven & Hurd 2012).
83 Flow rates directly impact the thickness of the DBL (Vogel 1999; Denny 2015) and variation
84 in metabolic processes can change the concentration gradients (Sand-Jensen, Revsbech &
85 Jørgensen 1985). In seaweeds, photosynthesis and respiration processes not only affect the
86 oxygen gradient of the DBL (e.g. Spilling *et al.* 2010) but they also change the pH due to the
87 uptake and release of DIC in light and dark, respectively (e.g. De Beer & Larkum 2001; Hurd
88 *et al.* 2011, Cornwall *et al.* 2013b ; Short, Pedersen & Kendrick 2015). This ability of
89 macrophytes to metabolically modify their local pH and the influencing effect of
90 hydrodynamics on DBL thickness have recently been put forward in the context of ocean
91 acidification (OA) to understand how seaweeds, seagrasses and their associated organisms,
92 particularly calcifiers, will respond to the predicted decrease in seawater pH, (Hendriks *et al.*
93 2015, 2017; Hurd 2015; Wahl, Saderne & Sawall 2016; Cox *et al.* 2017). The term OA
94 describes the average decrease in the surface oceanic pH and the associated changes in seawater
95 carbonate system caused by increasing CO₂ release into the atmosphere since the beginning of
96 the industrial revolution (Gattuso & Hansson 2011). There is no doubt that OA is an ongoing
97 process and that the pH will be reduced by 0.1 to 0.3 pH units by the end of the century (Stocker
98 *et al.* 2013), and it is therefore essential to find ways to increase the resilience of sensitive
99 species and discover temporal or spatial refuge from OA (Hurd 2015).

100 In this context, this study aims to better characterize the thickness and concentration
101 gradient of the DBL at the blade surface of the kelp *Ecklonia radiata*, (C.AGARDH) J.AGARDH
102 1848, one of the most common and widespread brown macroalgae in southern hemisphere
103 coastal oceans, which forms large and dense seaweed communities (Steneck *et al.* 2002). As
104 the interactive effect of flow rate, mainstream seawater pH and epiphytism on DBL have never
105 been characterized, we measured oxygen concentrations and pH values from the blade to the
106 mainstream seawater, in saturating light and dark conditions, in fast and slow flow, today's pH
107 and that predicted for 2100 and in the presence or absence of the common bryozoan
108 *Membranipora membranacea*, LINNAEUS 1767. We hypothesised that, 1. the DBL would be
109 thicker in slow flow because low velocity favours the increase of the DBL (Hurd 2000); 2. the
110 DBL would also be thinner for blades with bryozoa because the ciliary motion of the zooids
111 may create turbulence disturbing the laminar properties of the DBL; 3. the DBL oxygen
112 gradient would be thicker in future OA compared to current pH conditions because the
113 productivity of fleshy algae is expected to increase under OA (Kroeker *et al.* 2013); 4. oxygen
114 concentration will be lower on blades colonized by bryozoans than on bare blade as bryozoan
115 colonies can shade the tissue decreasing the intensity received and so the photosynthetic
116 efficiency of the blades (Muñoz, Cancino & Molina 1991). Finally, knowledge of oxygen and
117 pH fluctuations in these different conditions may give some clues on if DBL microenvironment
118 on kelp blades might be considered as refuge from OA because of the buffering effect of
119 seaweed metabolism to their surrounding pH, permitting temporal respite from very low
120 mainstream pH.

121

122 **Material and methods**

123 *Sample collection and laboratory acclimation*

124 Around 80 lateral blades from separate individuals of the kelp *Ecklonia radiata* were
125 collected using scissors, by snorkelling, in the Tinderbox reserve (-43.058177 S, 147.330749
126 E), close to Hobart (Tasmania, Australia) on 17th and 24th September 2015. Bare blades and
127 blades colonized by the bryozoan *Membranipora membranacea* were sampled each time.
128 Blades were similar in term of color and length, collected at the same depth (\approx 3-4 m) and the
129 bryozoan patches upon blades had a diameter of 3.1 ± 0.1 cm. Field collections were allowed
130 by permit provided by the Department of Primary Industries, Parks, Water and Environment of
131 Tasmania (n°15115). Blades freshly cut were transferred to the laboratory in a cool box to keep
132 them at the field temperature (13.5°C). They were then placed in a temperature controlled room
133 (13°C), in 0.1 μm filtered/UV sterilized seawater bubbled with air, under low light conditions
134 ($50 \mu\text{mol photons m}^{-2} \text{s}^{-1}$), until the beginning of the experiment, to acclimate them to laboratory
135 conditions.

136

137 *Experimental design*

138 All the experiments were carried out after a 2-day acclimation period, between 2 and 5
139 days after collection. Experiments were conducted in a 46 L unidirectional recirculating flume
140 (see description in Hurd *et al.* 1994b) filled with 0.1 μm filtered/UV sterilized seawater to a
141 depth of 15 cm. This type of unidirectional flume is considered as a standard tool to test the
142 interactions between organisms and their surrounding flow even if it cannot perfectly mimic
143 the hydrodynamics in the field (Vogel 1999). The flume was initially cleaned with a 1% sodium
144 hydroxide (bleach) solution and neutralized with a 1% thiosulfate solution (Hurd *et al.* 1994b).
145 It was subsequently rinsed with tap water every day, at the end of the daily experiments, to
146 avoid any biofilm formation inside. The flume was filled with seawater at the beginning of each
147 day and 8 different *Ecklonia* blade samples were measured over the same day.

148 Four blade replicates were measured in each different combination of light, mainstream
149 pH, flow and epiphytic condition i.e. bare blade or presence of bryozoans (64 different blades
150 in total). Measurements were carried out in both the light, at a saturating irradiance of 120 μmol
151 $\text{photons m}^{-2} \text{s}^{-1}$ provided by overhead lights (T8 840, 36W, Thorn Lighting, UK) and dark
152 conditions ($< 2 \mu\text{mol photons m}^{-2} \text{s}^{-1}$). Irradiance was measured with a flat underwater quantum
153 sensor LI-250A (LI-COR, Lincoln, USA). Two pH conditions were tested, the current sea-
154 surface average $\text{pH}_T = 8.09$ (hereafter 8.1) and a lower pH level predicted for the end of the
155 century in the worst-case scenario for ocean acidification, $\text{pH}_T = 7.71$ (hereafter 7.7) (Stocker
156 *et al.* 2013). The pH was adjusted by bubbling with 100% CO_2 into the seawater until reaching
157 the expected level (Hurd *et al.* 2011). Light and pH treatments were randomized in order to
158 have a single combination of light and pH per experimental day. In total, 8 days were necessary
159 to run the measurements for all the possible combined conditions.

160 Every day, two mainstream seawater velocities were randomly tested, a fast one, $> 8 \text{ cm}$
161 s^{-1} and a slow one, $< 0.5 \text{ cm s}^{-1}$. The flow characteristics in the different regimes were
162 determined in the middle of the flume using a field 10 MHz Acoustic Doppler Velocimeter
163 (Sontek, San Diego, CA, USA) before the start of the experiment. These velocities were chosen
164 because they represent speeds at which the DBL can reach maximal and minimal values
165 (Hansen, Hondzo & Hurd 2011) and they are similar to those observed within kelp beds in the
166 field (Jackson & Winant 1983; Gaylord *et al.* 2007; Kregting *et al.* 2011). Individual kelp blades
167 colonized or not by bryozoans were randomly assigned to the different flow treatments. Each
168 of the replicate blades were attached to an aluminium plate covered with plastic film so they
169 could not move during the trials. Measurements were always made at the top of a crenulation,
170 in the middle of the blade, in the middle of bryozoan patch (for the bryozoans present
171 condition), in the same position in the flume (Hurd & Pilditch 2011).

172

173 *Diffusive boundary layer measurements*

174 The characterization of the DBL, i.e. its thickness and the oxygen concentration and pH
175 gradient, was determined using a 50 μm O_2 micro-sensor and a 50 μm micro-pH electrode
176 coupled with a reference electrode (Unisense, Aarhus, Denmark), for 4 replicates in each
177 combined conditions of light, flow, bulk seawater pH and epiphytism (presence or absence of
178 bryozoans). The electrodes were attached to a Unisense MM33-2 manual micromanipulator
179 (Unisense, Aarhus, Denmark). A minimum period of 30 min acclimation to each treatment
180 occurred prior to starting vertical profiles, allowing the DBL to form and stabilize. Profiles of
181 O_2 concentration and pH were taken by placing the microelectrodes at the surface of the blade
182 and by sequentially increasing the height of the probes towards the mainstream seawater. The
183 measurements were made at 0.05, 0.1, 0.15, 0.2, 0.3, 0.4, 0.5, 0.6, 0.8, 1, 3, 5, 10 and 50 mm
184 (bulk seawater) above the blade surface, for 2 min each, logging O_2 and pH every second.
185 Values were then averaged over 30 seconds after the initial peak induced by the movement of
186 the electrode.

187 *DBL thickness:* DBL thickness was calculated for each O_2 profile, on the raw values,
188 and was defined as the greatest height above the surface of the blade at which the concentration
189 of O_2 was $<1\%$ per 0.1 mm for four subsequent measurements (Hurd *et al.* 2011; Cornwall *et*
190 *al.* 2015).

191 *O_2 profiles:* To describe the profiles, O_2 was first standardized by dividing the
192 concentration at any given profile location by the bulk seawater concentration measured at the
193 end of the profile. Profiles were then fitted and smoothed with an exponential equation: $y = y_0$
194 $+ \alpha \exp(-\beta x)$ with y : the standardized O_2 concentration, y_0 : a constant, α : the oxygen
195 standardized concentration when $x = 0$, β : the rate of change x : the distance from the blade.

196 *Interfacial oxygen fluxes*: net production in light and respiration in dark conditions were
197 defined as interfacial oxygen fluxes (Hofmann, Koch & de Beer 2016), calculated from the raw
198 concentration values of the profiles using Fick's first law (Revsbech & Jørgensen 1986):

$$199 \quad J = -D \left(\frac{dc}{dx} \right)$$

200 with J: interfacial oxygen fluxes in $\mu\text{mol O}_2 \text{ m}^{-2} \text{ s}^{-1}$, D: diffusion coefficient of oxygen in
201 seawater in $\text{m}^2 \text{ s}^{-1}$ ($D = 1.66 \cdot 10^{-9}$ at 13.2°C and salinity 37, value calculated with the R package
202 *marelac*), dc: the change in concentration in the DBL in $\mu\text{mol m}^{-3}$ and dx: the thickness of the
203 DBL in m.

204 *Data analyses*: DBL thicknesses, interfacial fluxes and O_2 standardized concentrations
205 at the surface of the blade (0 mm) were analyzed using 3-way ANOVAs with pH, flow and
206 presence/absence of bryozoan (factor called "blade") as crossed orthogonal factors followed by
207 post-hoc SNK tests. A 3-way MANOVA with the same factors was used to compare the
208 estimated y_0 , α and β coefficients of the curves fitted to the profiles. The analyses were run
209 independently for light and dark conditions, except for comparing DBL thicknesses in which
210 measurements in the light and dark conditions were combined because the DBL thickness is
211 affected by physical factors as flow speed and roughness but not light. All statistical analyses
212 were performed using the R software, version 2.15.0 (R Core Team 2013) after the normality
213 and the homoscedasticity of the data have been checked.

214 *pH profiles*: pH values acquired along the profiles were first transformed in H^+
215 concentration with the equation $[\text{H}^+] = 10^{-\text{pH}}$ (Riebesell *et al.* 2010). H^+ concentrations were
216 then standardized as for oxygen, by dividing the concentration at any given profile location by
217 the mainstream seawater concentration measured at the end of the profile. Standardized values
218 were then converted back in pH values using the equation $\text{pH} = -\log [\text{H}^+]$ to obtain profiles of
219 pH deviation from the mainstream pH in the DBL. Finally, linear regression was used to explore

220 the relationship between pH deviation from the mainstream value and the standardized oxygen
221 concentrations along the DBL.

222

223 *Seawater parameters*

224 During the eight experimental days, temperature, salinity, pH and nutrients were
225 measured three times a day, i.e. after flume filling, at midday and before flume draining.
226 Temperature was logged using a Precision Multi Digital thermometer (Testo, Lenzkirsh,
227 Germany), salinity was measured with a refractometer (HI96882, Hanna Instruments,
228 Woonsocket, RI, USA) and pH was determined immediately after sampling using a Orion star
229 A111 pH meter coupled with a OrionRoss ultra pH/ATC triode (Thermo Scientific, Waltham,
230 MA, USA) and calibrated with 4, 7 and 10 pH_{NBS} buffers. pH was standardized on the total
231 scale using TRIS buffers prepared as directed by (Dickson, Sabine & Christian 2007). Samples
232 for nutrients were frozen at -20°C until defrosted and analysed for nitrate (NO₃⁻) and phosphate
233 (PO₄⁻) using a QuickChem 8500 series 2 Automated Ion Analyzer (Lachat Instrument,
234 Loveland, CO, USA). Water for total alkalinity (A_T) was sampled once a day, pre-test showing
235 that alkalinity did not vary along the day. A_T samples were poisoned with mercuric chloride
236 (0.02% vol/vol; Dickson, Sabine & Christian 2007) pending analyses, made later at the
237 Australian National University, using an automatic built in-house titrator (consisting in a 5 mL
238 Tecan syringe pump (Cavro XCalibur Pump), a Pico USB controlled pH sensor, and a TPS pH
239 electrode). A_T values were then calculated using the Gran technique (Dickson, Sabine &
240 Christian 2007). Seawater carbonate chemistry, i.e. CO₂ partial pressure (*p*CO₂), carbon
241 dioxide, bicarbonate and carbonate ions concentrations ([CO₂], [HCO₃⁻] and [CO₃²⁻]), dissolved
242 inorganic carbon (DIC), and the saturation state of aragonite and calcite (Ω_{Ar} , Ω_{Ca}) were
243 calculated from temperature, salinity, pH and A_T values measured for each pH level with the

244 Seacarb package (Gattuso, Epitalon & Lavigne 2016) using constants from Mehrbach et al.
245 (1973) refitted by Dickson & Millero (1987).

246

247 **Results**

248 *Seawater parameters*

249 Average chemical parameters of the mainstream seawater at pH 8.1 and pH 7.7
250 conditions are given in Table 1. Standard errors of the values showed that the algae did not
251 strongly affect the bulk seawater chemistry, meaning that the physico-chemical parameters did
252 not vary during each daily experiment: the seawater was renewed each day. Seawater nutrients
253 of 3.4 μM for nitrate and ammonium and 1.3 μM for phosphate were within the normal range
254 for spring (Hepburn, Hurd & Frew 2006). According to the nutrient concentrations and DIC
255 values carbon or nitrogen limitation for photosynthesis are unlikely during our measurements.

256

257 *DBL thickness*

258 The average thickness of the DBL varied from 0.09 ± 0.03 mm (mean \pm SE) in fast flow,
259 pH 7.7 conditions on bare blades to 0.94 ± 0.30 mm in slow flow and pH 8.1 condition, in the
260 presence of bryozoans on the blade (Figure 1). While pH did not affect the thickness of the
261 DBL, the flow (3-way ANOVA, $df = 1$, $F = 25.908$, $p < 0.001$) and the presence of bryozoans
262 (3-way ANOVA, $df = 1$, $F = 5.976$, $p = 0.018$) had significant effects on its thickness. In slow
263 flow conditions, the DBL was always thicker than in fast flow conditions. Compared to bare
264 blades, the thickness of DBL was greater in blades colonized by bryozoans.

265

266 *O₂ profiles and interfacial fluxes*

267 In both light and dark conditions, O₂ profiles in fast flow treatments were much steeper
268 than in slow flow (Table 2, Gradient shape, p = 0.001 and 0.003 in light and dark conditions,
269 respectively), with initial oxygen concentration on the surface varying by 0.2 units (Figure 2 A,
270 B). Conversely, the oxygen gradients in slow flow treatments were more gradual, with a broader
271 range of O₂ values from varying by 0.6 units (Figure 2 C, D). Under dark conditions, the
272 presence of bryozoans decreased the oxygen content within the DBL to a greater extent than on
273 bare blades regardless of the flow speed and the mainstream pH (Table 2, Gradient shape, p =
274 0.003). Thus, the shape of the profiles differed between fast and slow flow and in the presence
275 or absence of bryozoans in dark conditions, but no interactive effects were detected either in
276 the light or dark.

277 The mean standardized O₂ concentration measured at the surface of the kelp blades in
278 the different conditions were differently affected by pH, flow and presence/absence of
279 bryozoans depending on the light conditions (Table 3). Under saturating light, O₂
280 concentrations were > 1 because photosynthetic processes released oxygen. So the oxygen
281 concentration just above the blade was higher than in the mainstream seawater. In the light, O₂
282 concentrations were significantly affected by pH (Table 2, surface [O₂], p = 0.044) with higher
283 concentrations in pH 7.7 condition (1.14 ± 0.03) compared to pH 8.1 (1.25 ± 0.06). O₂
284 concentrations were also significantly lower in fast than in slow flow (1.10 ± 0.03 and 1.28 ±
285 0.05, respectively, Table 2, surface [O₂], p = 0.002). In the dark, standardized O₂ concentrations
286 were < 1 because of the use of oxygen by respiration. In contrast to the light conditions, pH did
287 not affect the O₂ levels at the surface in the dark. However, similar to the light conditions, they
288 varied according to the flow, being higher in fast flow treatments than in slow flow (0.91 ± 0.02
289 and 0.59 ± 0.06, Table 2, surface [O₂], p > 0.001). The presence of bryozoans also significantly
290 decreased the oxygen concentrations at the surface compared to bare blades (0.68 ± 0.06 and

291 0.85 ± 0.05 , respectively, Table 2, surface $[O_2]$, $p = 0.008$). No interactive effects were detected
292 either in the light or dark.

293 Interfacial fluxes in light condition were assimilated to net production (Table 3). Net
294 production increased significantly by 80% in pH 7.7 compared to pH 8.1 (0.30 ± 0.05 and 0.17
295 $\pm 0.05 \mu\text{mol m}^{-2} \text{s}^{-1}$, respectively, Table 2, Interfacial flux, $p = 0.049$). The presence of
296 bryozoans also influenced the interfacial flux in the light (Table 2, Interfacial flux, $p = 0.042$).
297 Net production was higher on bare than epiphytized blades (0.29 ± 0.06 and $0.17 \pm 0.04 \mu\text{mol}$
298 $\text{m}^{-2} \text{s}^{-1}$, respectively), likely due to the oxygen consumption by bryozoans. No significant effect
299 of pH, flow or blade state on respiration rate, i.e. interfacial fluxes in dark, were detected.

300

301 *pH profiles and pH- O_2 relationship (slow flow conditions only)*

302 Among the 64 profiles measured, only 10 pH profiles in slow flow conditions were
303 useable due to technical issues with the microprobes. These profiles are presented in Figure 3,
304 which shows the variation in pH units from the mainstream seawater (increase in light
305 conditions and decrease in dark condition) for slow flow conditions, for bare blades and blades
306 with bryozoans, in the different mainstream pH conditions tested. On bare blades, pH variations
307 between light and dark were similar in both pH treatments, around 0.5 unit (Figure 3A).
308 However, pH at the blade surface in the pH 7.7 condition shifted towards higher values than
309 those measured in pH 8.1 treatment. In the light, the increase in photosynthesis at pH 7.7
310 elevated the mainstream pH by almost 0.3 units in the first μm of the DBL, compared to only
311 0.2 unit at pH 8.1. This means that the pH at the blade surface ranged from 7.8 to 8.3 in current
312 pH conditions and 7.5 to 8 in OA conditions, along daily cycles. For epiphytized blades at pH
313 8.1, the range of pH variation between light and dark was narrower than on bare blades (ΔpH
314 $= 0.26 \text{ pH}$, Figure 3B). At pH 7.7, pH fluctuations on blades with bryozoans varied depending
315 on the profiles, with narrower or larger fluctuations than for the pH 8.1 condition.

316 The relationship between pH deviation from the mainstream value and oxygen
317 standardized values in the DBL was linear for bare blades, in both light and dark conditions (R^2
318 = 0.95 to 0.99, $p < 0.01$, Figure 3C). The shape of the relationship for blades with bryozoans
319 were less obviously linear (Figure 3D) even if some of the linear regression were significant
320 ($R^2 = 0.82$ to 0.96).

321

322 **Discussion**

323 The fluctuations of oxygen concentrations and pH, occurring along DBL gradients in the
324 different conditions of light, flow, mainstream pH and epiphytism tested in our study showed
325 that interactions between abiotic and biotic factors can lead to unexpected chemical variations
326 in the microhabitats on the blades of the kelps and more generally on macrophytes (Koch 1994;
327 Short, Pedersen & Kendrick 2015; Hofmann, Koch & de Beer 2016). The presence of the
328 bryozoans on kelp blades increased the complexity of the habitat at the microscale. Particularly
329 in slow flow, DBL thickness increased, merging the algal DBL with that of the bryozoans'. The
330 interaction between the mainstream pH and the physiology of both kelp and epiphytes also led
331 to variations in oxygen concentrations and pH in the DBL which could afford protection from
332 future ocean acidification. This kind of interaction may enhance the resilience of organisms and
333 ecosystems to ocean acidification, even for those occurring on a microscale.

334 The thickness of the DBL was regulated by hydrodynamics, which engineer the laminar
335 layer above the blade where the chemical concentration gradient occurs. So, as we expected,
336 the DBLs were thin in faster flows, and thicker under slow flows. Seawater pH did not influence
337 the thickness of the DBL, in fast and slow flows, because physical processes set the maximum
338 thickness. The average thicknesses recorded for bare blades in our study for slow (~ 0.5 mm)
339 and fast flow (~ 0.1 mm) were within the range reported for *Macrocystis sp.*, a genus closely

340 related to *Ecklonia* and the only other kelp for which DBL thickness have been measured (Hurd
341 2000 and references therein) and thinner (-50%) than those of coralline algae (Raven & Hurd
342 2012).

343 The thickness of the DBL was not only a factor of flow speed but was also affected by the
344 presence of bryozoans on *E. radiata* blades. Opposite to our second hypothesis, the DBL was
345 thicker on blades colonized by bryozoans (~ 0.8 mm in slow flow) than on bare blades (~ 0.3
346 mm in fast flow). The ciliary motion of zoides was expected to create turbulence, impacting the
347 building of the laminar layer above the blade. However, the presence of the bryozoan layer
348 increased the thickness of the DBL in all the conditions by creating their own DBL in addition
349 to the kelp's one, essentially as a small canopy boundary layer (Cornwall et al. 2015). This
350 phenomenon has also been observed on *Fucus* species where the presence of hyaline hairs
351 increased the DBL thickness compared to hairless thalli (Spilling *et al.* 2010; Lichtenberg,
352 Nørregaard & Kühl 2017). The explanation is that small scale (μm - mm) surface topographical
353 features such as corrugations, the presence of hyaline hairs or the cilia of bryozoans likely
354 reduce the roughness Reynold's number (Hurd *et al.* 1997; Hurd & Pilditch 2011; Lichtenberg,
355 Nørregaard & Kühl 2017) or create a local depression (Wangpraseurt *et al.* 2012) thus
356 increasing the DBL thickness. These engineering factors, flow velocity and the presence of
357 bryozoans, are therefore not only able to affect the thickness of the DBL but also can directly
358 and/or indirectly impact the chemical gradients occurring therein.

359 Measuring the oxygen gradient occurring in the DBL is a good way to understand and
360 characterize the surface micro-chemistry and how variable these fine-scale environments can
361 be (Shashar, Cohen & Loya 1993). Flow was the main factor which influenced the O₂ gradients
362 within the DBL at the surface of *E. radiata*. However, net photosynthesis was similar in fast
363 and slow flow at the surface of *E. radiata*, suggesting that the photosynthetic process was not
364 mass-transfer limited by the thick DBL in fast slow flow conditions. This finding is similar to

365 that found for inorganic nitrogen uptake by the kelp *Macrocystis pyrifera* (Hurd et al. 1996)
366 while other studies report mass transfer limitation of photosynthesis (Carpenter & Williams
367 2007; Mass *et al.* 2010). As the DBL was very thin in fast flow, the profiles were very steep
368 and sharp in light and dark conditions and the standardized oxygen values were lower than in
369 slow flow because of fast diffusion of the oxygen molecules out of the DBL (Irwin & Davenport
370 2002; Irwin & Davenport 2010). Thus, the main differences between the other parameters
371 (mainstream pH, epiphytism) were better compared in slow flow conditions, as follows.

372 Net photosynthesis measured on blades increased with the pH decrease predicted for the
373 end of the century, in both bare and epiphytized blades while respiration remained similar in
374 both pH conditions. At pH 7.7, the standardised oxygen values measured in the light were
375 greater in the first 0.2 mm layers of the gradient, close to the kelp surface, than at pH 8.1, due
376 to the increase in photosynthesis. Because *E. radiata* is a HCO_3^- -user for photosynthesis
377 (Falkenberg, Russell & Connell 2013; Cornwall, Reville & Hurd 2015; Britton *et al.* 2016), the
378 greater CO_2 availability related to the pH decrease may not benefit the photosynthesis process
379 (Koch *et al.* 2013, Britton *et al.* 2016), as shown for *Macrocystis pyrifera* (Fernández, Roleda
380 & Hurd 2015). However the conversion of HCO_3^- to CO_2 , the substratum required by the
381 enzyme RuBisCO, implies carbon concentrating mechanism(s) (CCM) which require more
382 energy than the passive diffusion of CO_2 (Giordano, Beardall & Raven 2005; Raven & Beardall
383 2014). Thus, the increase in both CO_2 availability and passive diffusion at pH 7.7 may provide
384 an advantage to *E. radiata*, reducing energetic costs of CCMs and helping to increase primary
385 production (Cornwall *et al.* 2012; Raven, Beardall & Giordano 2014).

386 The presence of bryozoans on the kelp blades was correlated to a decrease in the
387 standardized O_2 concentrations in the DBL compared to bare blades. Net photosynthesis was
388 lower on epiphytised blades as the bryozoans likely used oxygen produced by the algae for
389 respiration. Bryozoans might also have caused shading on the algal blade, decreasing its

390 photosynthetic efficiency (Muñoz, Cancino & Molina 1991). However, no change in
391 chlorophyll *a* content was detected between bare and epiphytised blades (see Supporting
392 Informations), supporting the findings of prior studies that reveal a lack of bryozoan effect on
393 pigment acclimation of kelp blades, and thus on photosynthetic efficiency (Hepburn, Hurd &
394 Frew 2006). The bryozoans' contribution to the oxygen concentration gradient was particularly
395 obvious in the dark at pH 8.1, where standardized oxygen values of blades colonized by
396 bryozoans were two-times lower than on bare blades. This trend was less pronounced at pH 7.7
397 in the dark whereby the DBL oxygen concentrations looked similar for epiphytized and bare
398 blades. The pH decrease in the mainstream seawater did not affect the respiration rates of the
399 epiphyte/blade complex at the blade surface likely because bryozoans present a great plasticity
400 and different strategies which enable them to cope with pH decrease (Swezey *et al.* 2017a;
401 Swezey *et al.* 2017b). Moreover, the pH fluctuations occurring in the micro-environment of
402 seaweeds can reach very low levels, from 8.1 down to 7.0 in the dark (De Beer & Larkum 2001;
403 Hurd *et al.* 2011) and bryozoans living upon the blades may be used to these daily drops in the
404 surrounding pH.

405 It is more and more recognized that the biological activity of both the seaweeds and their
406 epibionts can generate pH fluctuations with ranges, rates and magnitude of change that mask
407 the long-term trend predicted for the open ocean (Krause-Jensen *et al.* 2015; Hendriks *et al.*
408 2017; Wahl *et al.* 2017). pH measured at the surface of bare blades of *E. radiata* was linearly
409 correlated to the oxygen values recorded in the DBL in light as in dark conditions, as it has been
410 shown for other seaweeds (De Beer & Larkum 2001; Larkum, Koch & Kühl 2003; Cornwall *et*
411 *al.* 2013b). In slow flow, the mean daily pH in the first layers of the DBL remained higher in a
412 mainstream pH 8.1 compared to 7.7, similar to the findings for coralline algae (Cornwall *et al.*
413 2013b). However, pH fluctuations occurring in the DBL at low mainstream pH could reach pH
414 values > 7.7 in the light, likely providing respite conditions from the corrosive mainstream

415 seawater. This rise in pH at the blade surface compared to the bulk seawater pH has also been
416 measured in other macrophytes, with an increase from 0.4 to 1.2 pH unit (Hendricks *et al.*
417 2017). In our study, the presence of bryozoans narrowed the pH range experienced at the blade
418 surface in pH 8.1 condition compared to bare blades, restricting the exposure of bryozoans to
419 low pH values. In low pH conditions, however, the intensity of DBL pH fluctuation differed
420 depending on the profiles, maybe because of individual-specific responses of bryozoans to pH
421 due to phenotypic variance (Eriander, Wrangé & Havenhand 2015). Overall, the shift in the
422 range of pH in the DBL towards higher values than the bulk seawater and narrower fluctuations
423 in the presence of bryozoans could lead to local chemical conditions which may be more
424 favorable than those of the mainstream seawater for organisms living on top or under the blades.
425 Thus, DBL microhabitats may constitute a refuge from the general pH decline predicted in the
426 context of ocean acidification. Such variations have also been evidenced at the blade surface of
427 green algae and seagrasses (Hendricks *et al.* 2017), extending the concept of OA refuge for
428 small and understory organisms to other macrophytes.

429 The substantial fluctuations in O₂ concentration and pH reported here in the DBL and in
430 other studies on macrophytes (e.g. Hofmann *et al.* 2016; Hendrick *et al.* 2017) may represent
431 an alternation between stressful periods and release from pH stress for organisms associated
432 with seaweeds (Shashar, Cohen & Loya 1993; Wahl, Saderne & Sawall 2016). Living in
433 microhabitats with high fluctuations of environmental variables would be particularly
434 interesting for calcifying organisms in the context of ocean acidification. In fluctuating
435 environments, calcifiers may be less exposed, at least for a shorter period (Wahl *et al.* 2015), to
436 corrosive low pH/carbonate chemistry parameters which might decrease the dissolution process
437 and help them to cope with ocean acidification (Cornwall *et al.* 2014; Wahl *et al.* 2017).
438 Calcifiers such as mussels are able to take advantage of these fluctuations by shifting the
439 majority of their costly physiological processes, including calcification, to times when the

440 surrounding chemical conditions are more favorable (i.e. daytime) (Wahl *et al.* 2017). The DBL
441 chemical microhabitat could also constitute refuge and benefit other small invertebrates for
442 whom ocean acidification can affect the regulation of cellular homeostasis (Melzner *et al.*
443 2009). They might then represent interesting and attractive settlement sites for early life stage
444 of invertebrates which are supposed to lack efficient acid-base regulation systems (Byrne 2011).

445 Organisms which regularly encounter such strong fluctuations might be better able to
446 survive in the lower average pH seawater of the future, because they may present higher
447 phenotypic plasticity (Hurd *et al.* 2011; Boyd *et al.* 2016). Transient transgressions of tolerance
448 thresholds followed by relaxation periods in fluctuating regimes may not only favor high
449 phenotypic plasticity but may also select for more robust genotypes (Melzner *et al.* 2009;
450 Frieder *et al.* 2014). Thus the microenvironment of the DBL, particularly in slow flows, may
451 act as a stop-gap, enabling calcifiers and other organisms to acclimatize or adapt to lower pH
452 conditions projected for the coming decades (Hurd 2015).

453 The concept of refuge highlighted in this study can be generalized for the DBL
454 environments at the surface of kelp blades towards larger scales under the influence of biogenic
455 fluctuations created by macrophytes. Different studies have shown that oxygen and pH
456 fluctuations recorded in seagrass meadows (Unsworth *et al.* 2012; Hendriks *et al.* 2014; Saderne
457 *et al.* 2015) and seaweeds beds (Buapet, Gullström & Björk 2013; Pajusalu *et al.* 2013; Wahl
458 *et al.* 2017) were masking the global pH decrease predicted in the context of OA. These specific
459 chemical habitats dominated by macrophytes could help in mitigating the negative impact of
460 ocean acidification on calcifiers which live on the blades, in the canopy and in understory
461 (Semesi, Beer & Bjork 2009; Cox *et al.* 2017). They would not only provide temporal and
462 spatial refuge, enabling species to better cope with stressful conditions, but they could also
463 constitute selective areas facilitating the hardening of some populations and then, helping in
464 conserving resistant locally adapted populations.

465

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474

475 **Author contribution:**

476 FN and CH conceived and designed the experiments. FN performed the experiments and
477 analyzed the data. FN and CH wrote the manuscript.

478

479 **Data accessibility**

480 All data from this article will be available on Pangaea database, under OA-ICC data
481 compilation, with a digital object identifier DOI assigned later.

482

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754 **Supporting information**

755 Additional supporting information about chlorophyll contents may be found in the online
 756 version of this article.

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759 **Table 1:** Seawater parameters in current and low pH conditions. Temperature, salinity, pH_T,
 760 total alkalinity (A_T) and nutrients were measured directly in the surrounding seawater while all
 761 the other parameters of the carbonate chemistry (pCO₂, [CO₂], [HCO₃⁻], [CO₃²⁻], DIC, Ω_{Ar} and
 762 Ω_{ca}) were calculated.

	pH 8.1 condition			pH 7.7 condition		
	n	Mean	SE	n	Mean	SE
Temperature (°C)	13	13.19 ±	0.12	12	13.17 ±	0.12
Salinity	13	36.85 ±	0.10	12	37.25 ±	0.25
pH _T	13	8.09 ±	0.02	12	7.71 ±	0.01
A _T (μEq kg ⁻¹)	5	2099.82 ±	26.48	5	2242.72 ±	16.93
pCO ₂ (μatm)	13	330.87 ±	16.81	12	932.33 ±	22.89
[CO ₂] (μmol kg ⁻¹)	13	12.92 ±	0.65	12	36.34 ±	0.86
[HCO ₃ ⁻] (μmol kg ⁻¹)	13	1712.79 ±	12.69	12	2049.86 ±	4.17
[CO ₃ ²⁻] (μmol kg ⁻¹)	13	151.58 ±	4.97	12	76.56 ±	1.62
DIC (μmol kg ⁻¹)	13	1877.29 ±	8.36	12	2162.76 ±	3.41
Ω _{Ar}	13	2.29 ±	0.08	12	1.15 ±	0.02
Ω _{ca}	13	3.57 ±	0.12	12	1.80 ±	0.04
[NH ₄] (μM)	12	3.03 ±	0.37	12	3.51 ±	0.11
[NO ₃], [NO ₂] (μM)	13	3.64 ±	0.14	12	3.15 ±	0.22
[PO ₄] (μM)	13	1.26 ±	0.01	12	1.26 ±	0.02

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766 **Table 2:** Statistical results from 3-way ANOVA and MANOVA investigating the simple and
767 interactive effects of the mainstream pH (8.1 or 7.7), the flow speed (fast or slow), and the state
768 of the blade (bare or with bryozoans) on interfacial O₂ fluxes, O₂ standardized concentrations
769 at the blade surface and DBL O₂ gradient parameters (i.e. y₀, α and β from the fitted exponential
770 growth shape curve). Number in bold indicate significant effects.

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		Interfacial flux		Surface [O ₂]		Gradient shape (y ₀ , α and β from fitted equation)	
		<i>3-way ANOVA</i>		<i>3-way ANOVA</i>		<i>3-way MANOVA</i>	
	Df	F-value	p-value	F-value	p-value	F-value	p-value
pH	1	4.435	0.049	4.560	0.044	1.403	0.273
Flow	1	0.018	0.895	12.730	0.002	8.117	0.001
Blade	1	4.747	0.042	0.597	0.448	0.414	0.745
pH:Flow	1	0.251	0.622	1.681	0.208	0.837	0.490
pH:Blade	1	0.326	0.575	0.004	0.952	0.656	0.589
Flow:Blade	1	2.782	0.112	3.320	0.082	0.731	0.546
pH:Flow:Blade	1	0.430	0.520	1.256	0.275	0.950	0.436
	Df	F-value	p-value	F-value	p-value	F-value	p-value
pH	1	1.310	0.268	0.453	0.508	1.150	0.356
Flow	1	2.102	0.165	30.088	>0.001	6.668	0.003
Blade	1	1.668	0.214	8.598	0.008	6.684	0.003
pH:Flow	1	0.773	0.392	0.002	0.962	0.504	0.684
pH:Blade	1	2.009	0.174	3.356	0.081	2.174	0.126
Flow:Blade	1	0.054	0.819	1.319	0.264	2.449	0.097
pH:Flow:Blade	1	0.054	0.819	1.624	0.217	0.857	0.481

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777 **Table 3:** Mean (\pm SE) standardized O₂ concentrations and interfacial fluxes (J in $\mu\text{mol O}_2 \text{ m}^{-2}$
778 s^{-1}) at the surface of the blade in the different experimental conditions of pH, flow,
779 presence/absence of bryozoans and in saturated light and dark conditions. N = 3 or 4.

Mean standardized O ₂ concentration at the blade surface						
pH	Flow	Blade state	Light condition		Dark condition	
8.1	Fast	Bare	1.07	0.04	0.96	0.02
8.1	Fast	Bryozoans	1.08	0.06	0.81	0.04
8.1	Slow	Bare	1.24	0.06	0.81	0.11
8.1	Slow	Bryozoans	1.16	0.09	0.39	0.09
7.7	Fast	Bare	1.05	0.05	0.97	0.02
7.7	Fast	Bryozoans	1.17	0.07	0.90	0.05
7.7	Slow	Bare	1.49	0.14	0.65	0.09
7.7	Slow	Bryozoans	1.29	0.07	0.60	0.14

Interfacial flux J ($\mu\text{mol m}^{-2} \text{ s}^{-1}$)						
pH	Flow	Blade state	Light condition		Dark condition	
8.1	Fast	Bare	0.16	0.12	-0.18	0.05
8.1	Fast	Bryozoans	0.13	0.10	-0.33	0.05
8.1	Slow	Bare	0.27	0.07	-0.23	0.14
8.1	Slow	Bryozoans	0.09	0.08	-0.37	0.07
7.7	Fast	Bare	0.31	0.09	-0.15	0.11
7.7	Fast	Bryozoans	0.31	0.02	-0.17	0.07
7.7	Slow	Bare	0.47	0.13	-0.31	0.06
7.7	Slow	Bryozoans	0.16	0.05	-0.28	0.05

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787 **Figure 1:** DBL thickness in mm in fast (on the left) and slow (on the right) flow conditions, in
788 pH 8.1 (white bars) and pH 7.7 (grey bars), in the absence (plain bars) or presence of bryozoans
789 (striped bars). Values are mean \pm SE, n = 6 or 8.

790

791 **Figure 2:** O₂ standardized profiles in fast (2 graphs on the left) and slow (2 graphs on the right)
792 conditions, in pH 8.1 (2 graphs on the top) and pH 7.7 (2 graphs on the bottom). White symbols
793 are for O₂ concentrations measured in saturating light while black ones are for dark
794 measurements. Circle represent measurements on bare blade while triangles are for blades
795 colonized by bryozoans. Values are mean \pm SE, n = 3 or 4

796

797 **Figure 3:** pH variations and relationship with oxygen concentration in the DBL. Individual pH
798 profiles in slow flow conditions are shown for bare blades (graph A, 4 profiles) and blades
799 colonized by bryozoans (graph B, 6 profiles). Solid lines represent pH deviation from
800 mainstream pH condition for pH 8.1 and dashed ones for pH 7.7, in both light (grey lines) and
801 dark (black lines) conditions. Graphs C and D present the relationship between pH variation
802 and oxygen standardized concentrations in the DBL for bare blades and blades colonized by
803 bryozoans, respectively. Data are from the same individual profiles as graphs A and B, with
804 grey and black symbols for light and dark conditions, respectively. Circles are for pH_T 8.1 as
805 both triangles show data for pH_T condition 7.7.

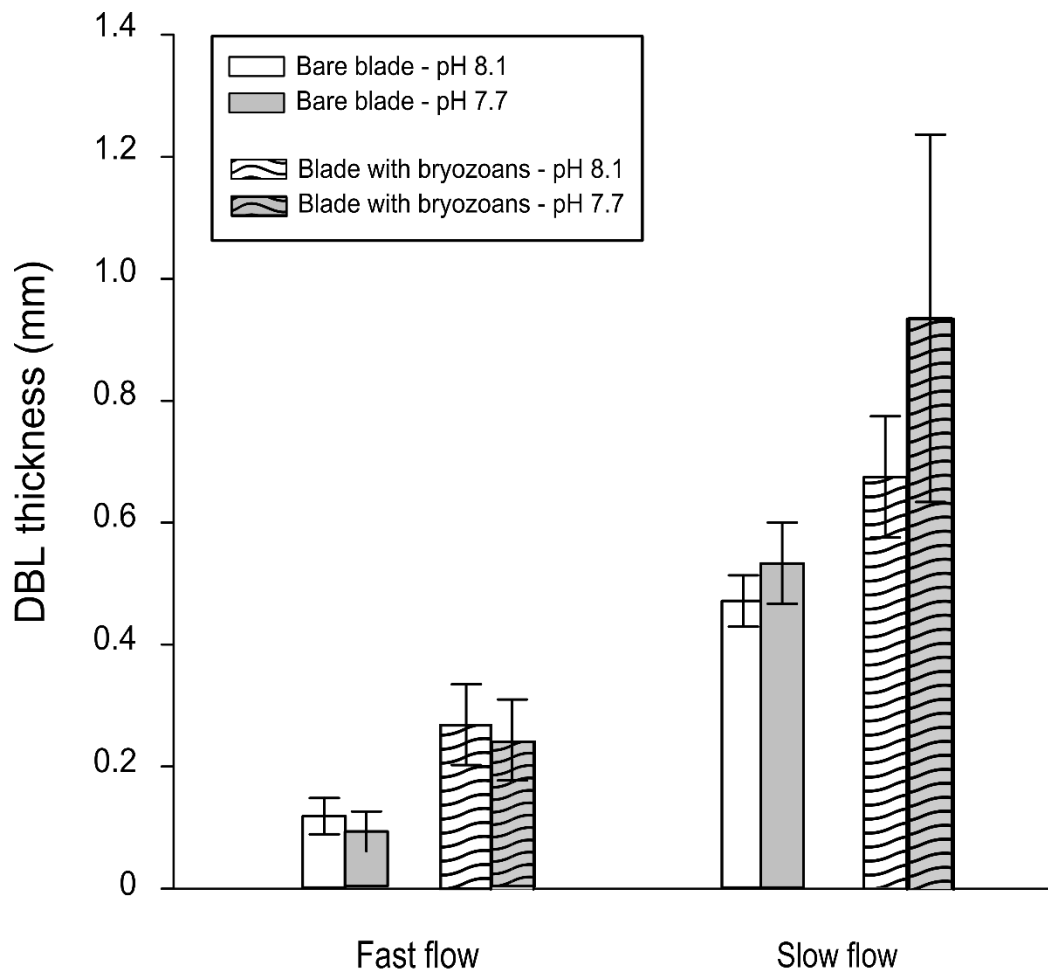
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810 Figure 1



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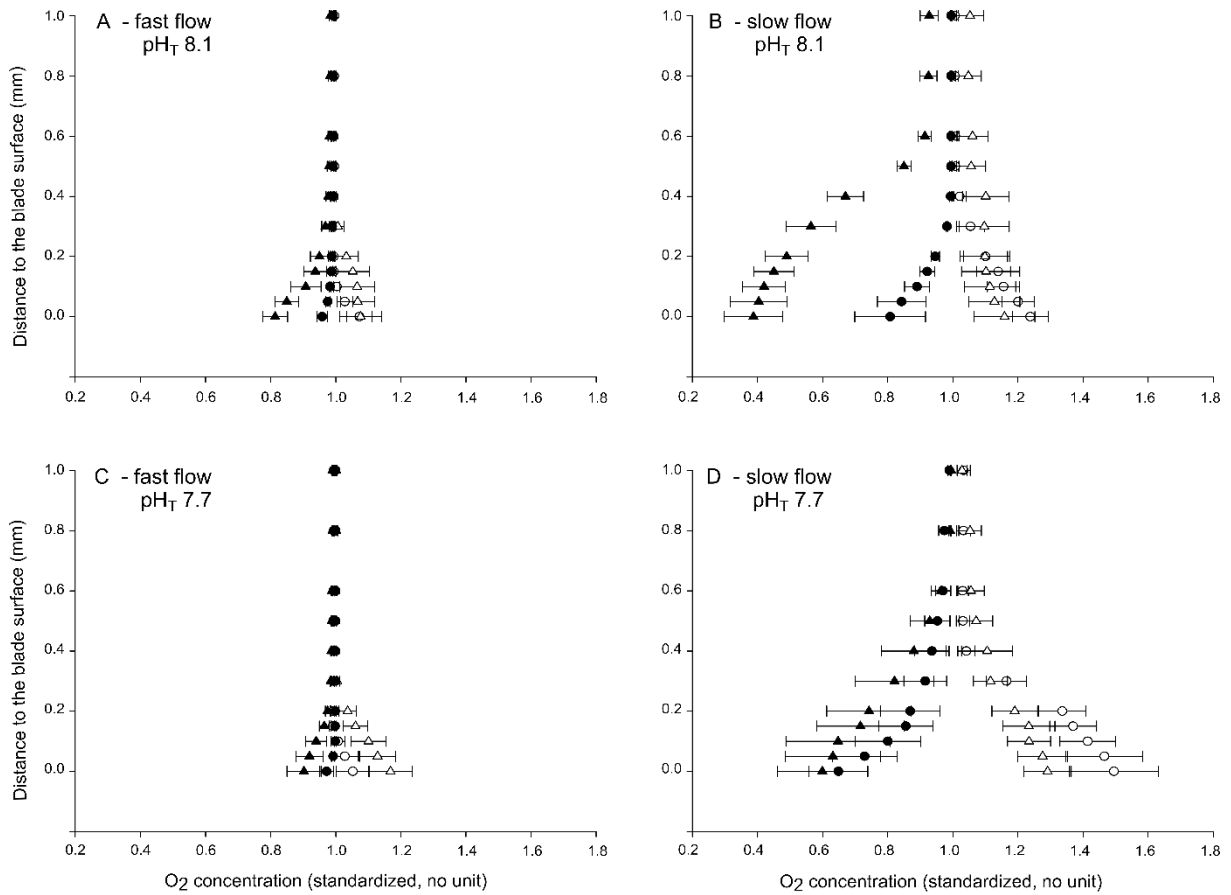
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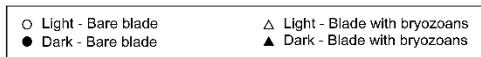
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819 Figure 2



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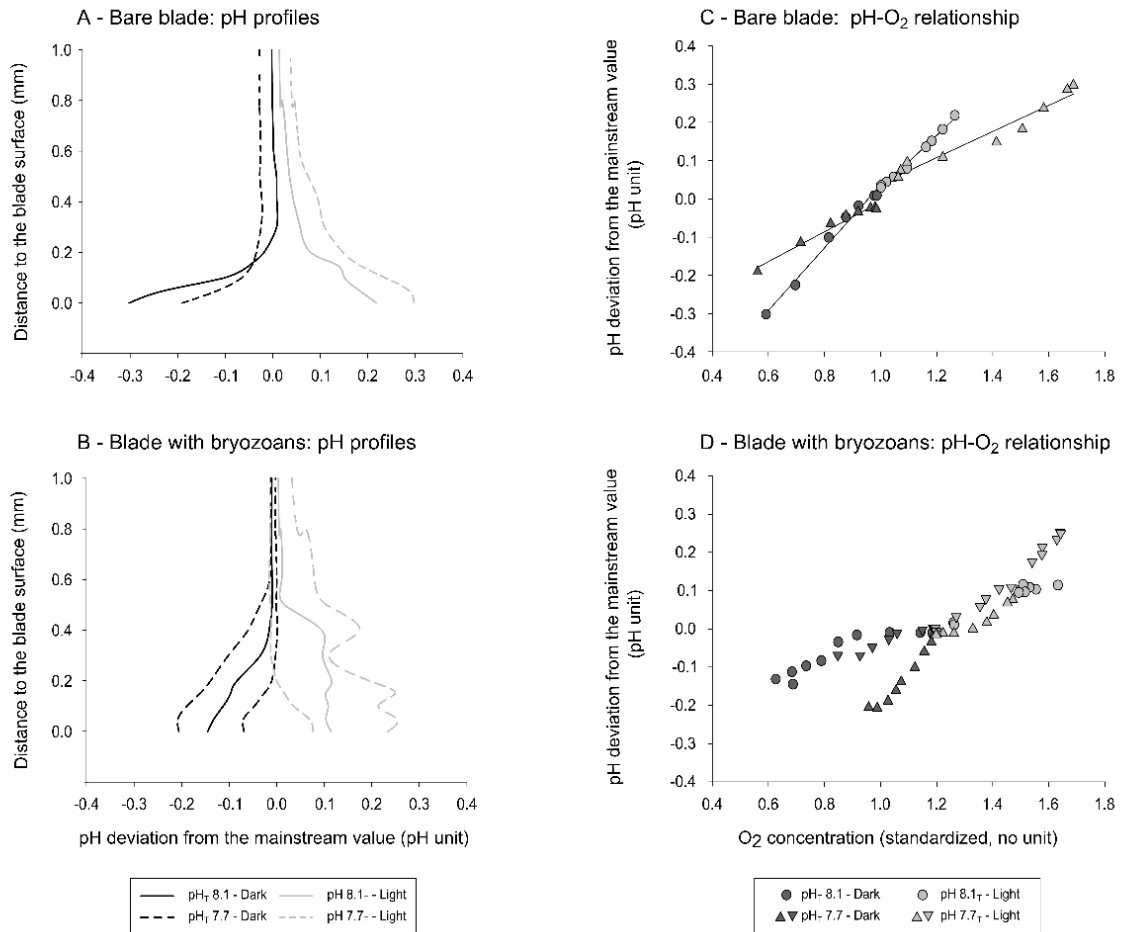
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