

1 **Authors:** James R. Collins^{1,2*†}, Paul D. Fucile³, Glenn McDonald⁴, Justin E. Ossolinski², Richard G.
2 Keil⁵, James R. Valdes³, Scott C. Doney², and Benjamin A. S. Van Mooy^{2*}

3 **Title:** An autonomous, *in situ* light-dark bottle device for determining community respiration and net
4 community production

5 **Affiliations:** ¹ MIT/WHOI Joint Program in Oceanography/Applied Ocean Science and Engineering,
6 Woods Hole, Massachusetts, USA

7 ² Department of Marine Chemistry and Geochemistry, Woods Hole Oceanographic Institution,
8 Woods Hole, Massachusetts, USA

9 ³ Department of Physical Oceanography, Woods Hole Oceanographic Institution, Woods Hole, MA
10 02543, USA

11 ⁴ Department of Applied Ocean Physics and Engineering, Woods Hole Oceanographic Institution,
12 Woods Hole, MA 02543, USA

13 ⁵ School of Oceanography, University of Washington, Seattle, WA 98195, USA

14 *** Correspondence:** James R. Collins, james.r.collins@aya.yale.edu or Benjamin A. S. Van Mooy,
15 bvanmooy@whoi.edu

16 **† Present Address:** James R. Collins, School of Oceanography and eScience Institute, University of
17 Washington, Seattle, WA 98195, USA

18 **Running head:** Autonomous observations of community metabolism

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20 instrumentation, optodes, dissolved oxygen, ocean observing

21

22 **Abstract**

23 We describe a new, autonomous, incubation-based instrument that is deployed *in situ* to
24 determine rates of gross community respiration and net community production in marine and aquatic
25 ecosystems. During deployments at a coastal pier and in the open ocean, the PHORCYS
26 (*PH*Otosynthesis and *R*espiration *C*omparison-*Y*ielding *S*ystem) captured dissolved oxygen fluxes
27 over hourly timescales that were missed by traditional methods. The instrument uses fluorescence-
28 quenching optodes fitted into separate light and dark chambers; these are opened and closed with
29 piston-like actuators, allowing the instrument to make multiple, independent rate estimates in the
30 course of each deployment. Consistent with other studies in which methods purporting to measure
31 the same metabolic processes have yielded divergent results, respiration rate estimates from the
32 PHORCYS were systematically higher than those calculated for the same waters using a traditional
33 two-point Winkler titration technique. However, PHORCYS estimates of gross respiration agreed
34 generally with separate incubations in bottles fitted with optode sensor spots. An Appendix describes
35 a new method for estimating uncertainties in metabolic rates calculated from continuous dissolved
36 oxygen data. Multiple successful, unattended deployments of the PHORCYS represent a small step
37 toward fully autonomous observations of community metabolism. Yet the persistence of unexplained
38 disagreements among aquatic metabolic rate estimates — such as those we observed between rates
39 calculated with the PHORCYS and two existing, widely-accepted bottle-based methods — suggests
40 that a new community intercalibration effort is warranted to address lingering sources of error in
41 these critical measurements.

42

43

44 **Introduction**

45 Accurate, reproducible, and cost-effective estimates of aerobic respiration and primary
46 production in aquatic systems are essential for research across a diverse array of disciplines in the
47 environmental sciences (del Giorgio and Williams, 2005; Volkmar and Dahlgren, 2006; Staehr et al.,
48 2012). Rate measurements of these two metabolic parameters can be applied to various problems,
49 including validating the biogeochemical components of global climate models (Denman et al., 2007),
50 determining the trophic status of surface-water planktonic communities in open ocean ecosystems
51 (Williams, 1998), measuring rates of biological oxygen demand (BOD) in treated wastewater
52 (Spanjers et al., 1994), and identifying unexpected metabolisms in the deep ocean (Reinthalter et al.,
53 2010).

54 While an increasing demand for metabolic rate data has encouraged the development of many
55 different methods for estimating rates of photosynthesis (Ducklow and Doney, 2013), the number of
56 new methods for measuring aerobic respiration at the community scale has lagged behind
57 considerably (del Giorgio and Williams, 2005). The majority of field-based methods for measuring
58 rates of respiration and primary production in the ocean fall largely into two categories: (1) *in situ*
59 geochemical tracer techniques that track changes in the concentration or isotopic composition of
60 dissolved oxygen and carbon dioxide within ocean water masses (e.g., the surface mixed layer), and
61 (2) *in vitro* incubation techniques that track the rates at which plankton exchange oxygen or carbon
62 dioxide in discrete seawater samples (i.e., bottle incubations). The merits and faults of these two
63 categories of approaches have been vigorously debated while significant and often unexplained
64 differences are noted in the rate estimates they yield (Duarte et al., 2013; Ducklow and Doney, 2013;
65 Williams et al., 2013). The former category has benefited considerably from recent advances in
66 optical sensor technology (Moore et al., 2009), mass spectrometry (Goldman et al., 2015), and
67 techniques for analysis of optical sensor data from autonomous underwater vehicles (Nicholson et al.,

68 2015). By maximizing the extent to which sensors are integrated into the surrounding environment,
69 low-power instruments increase the spatial and temporal resolution of geochemical tracers *in situ* and
70 permit increasingly autonomous, long-term deployments (Prien, 2007; Riser and Johnson, 2008;
71 Porter et al., 2009).

72 By contrast, the field has seen relatively few technical advances in *in vitro* incubation
73 techniques. *In vitro* techniques provide an important complement to *in situ* methods because they are
74 sensitive to short-term perturbations and are amenable to experimental design. For these reasons, the
75 traditional two-point light and dark bottle incubation technique (Gaarder and Gran, 1927) and the ^{14}C
76 incubation method (Steeman Nielsen, 1952) continue to dominate incubation-based studies, although
77 a number of other methods based on electron transport (e.g., Kenner and Ahmed, 1975) or fluxes of
78 ^{18}O or CO_2 (Bender et al., 1987; Robinson and Williams, 2005) have been introduced over the course
79 of the last half-century. A number of these methods have been incorporated into modern designs for
80 benthic flux chambers and so-called “benthic landers,” enabling investigators to capture fluxes of
81 oxygen and other gases *in situ* at the sediment-water interface instead of in core samples aboard ship
82 (Hammond et al., 2004; compare, e.g., Martens et al., 2016, Fuchsman et al., 2015, or Lee et al.,
83 2015, to Kim et al., 2015). However, even the most advanced of these devices can require the use of
84 divers or remotely operated vehicles (ROVs) for deployment, maintenance, or recovery.
85 Additionally, by their nature, few of these designs can be programmed to conduct multiple
86 incubations over the course of a single deployment. Taylor et al. addressed this obstacle with the
87 submersible incubation device (SID), which for the first time allowed multiple, unattended
88 incubations with ^{14}C -bicarbonate to be conducted *in situ* (Taylor and Doherty, 1990; Taylor et al.,
89 1993). The SID represented a significant advance but was limited by its relatively small 400 mL
90 incubation chamber and its reliance on the use of radiolabeled reagents.

91 Among the advances most consequential for *in situ* instrumentation was the adaptation to
92 marine applications of optical technologies such as optodes (e.g., Klimant et al., 1995; Tengberg et
93 al., 2006) and optode sensor spots (e.g., Warkentin et al., 2007), which exploit fluorescence
94 (luminescence) quenching to measure dissolved oxygen concentrations non-destructively and without
95 themselves contributing to oxygen consumption in the sample. Integral optodes and sensor spots
96 based on the same technology have now been successfully used in a variety of shipboard
97 configurations to measure rates of gross community respiration in whole, unconcentrated and
98 unfiltered water samples and in water containing particle material from marine and aquatic
99 environments (Edwards et al., 2011; Wikner et al., 2013; Collins et al., 2015). More recently,
100 shipboard *in vitro* measurements of respiration within individual marine particles were made
101 successfully using oxygen microelectrodes (Belcher et al., 2016a; Belcher et al., 2016b). A
102 significant recent advance was also achieved with the RESPIRE device, which uses an optode fitted
103 into a modified sediment trap to make particle respiration measurements *in situ* (Boyd et al., 2015;
104 McDonnell et al., 2015).

105 Despite the significant progress represented in these optode-driven systems, incubation-based
106 methods remain prone to a number of sources of error that demand reconciliation. These can be
107 generally divided into two categories: (1) those that result from the preparation for or act of
108 incubating natural microbial populations and (2) errors inherent in the method used to determine the
109 concentration of dissolved oxygen (e.g., Winkler titration, fluorescence-quenching optode, or Clark
110 electrode). The sources of uncertainty associated with bottle/chamber incubations span both
111 categories and include (1) contamination, disruption, or bias introduced through the process of
112 obtaining seawater samples from depth and preparing them for incubation (Suter et al., 2016;
113 Tamburini et al., 2013); (2) unrepresentative incubation conditions that do not faithfully reproduce
114 the variations in temperature, turbulence, and light inherent in natural systems; (3) so-called “bottle

115 effects" associated with low-volume incubations which may limit nutrient availability (Furnas, 2002)
116 or induce unnatural changes in community structure (Venrick et al., 1977; Calvo-Díaz et al., 2011);
117 and (4), in the case of metabolic rate measurements extrapolated from Winkler (1888) titrations, the
118 lack of temporal resolution inherent in measurements based only on two endpoints. In any study
119 where incubations are used, the choice of incubation methodology places inherent limits on the
120 spatial and temporal resolution of the data collected (Karl et al., 2001). The integration of point
121 measurements — data sparse in time and/or space, whether based on *in situ* observations or
122 incubations — also creates significant representation error. One solution to this problem is to greatly
123 increase the number of measurements made during data collection using automated technologies.

124 We describe here the *PHO*tosynthesis and *Respiration* Comparison-Yielding System
125 (PHORCYS), a large-volume (i.e., > 2.5 L), light and dark chamber incubation system for
126 autonomous measurement of rates of primary production and respiration at high temporal resolution
127 and under *in situ* conditions. In designing the instrument, we endeavored to minimize the major
128 hypothesized sources of uncertainty associated with traditional incubation-based methods while
129 constructing a system that functions autonomously and interrogates water samples non-destructively.
130 We also sought to eliminate or reduce the need for repeated wet-chemical field measurements such as
131 Winkler oxygen titrations or reagent-based methods used in other autonomous systems. We first
132 describe design and validation of the PHORCYS using two independent methods, and then present
133 results of several deployments of the instrument in different ecosystem types.

134 **Materials and procedures**

135 *Instrument design and operation*

136 The PHORCYS is composed of only a few basic components, making the design highly
137 scalable and cost-effective (Fig. 1a,b). In nearly all instances, "off the shelf" components of different

138 size or capacity can be easily substituted for those we describe here. The PHORCYS consists of two
139 polycarbonate plastic chambers (usable vol. 5.7 L; Table 1), several auxiliary sensors for collection
140 of environmental data in the ambient water mass outside the chambers, and a watertight power
141 supply, control, and data recording module (Fig. 1a). A piston-like, magnetically coupled actuator is
142 programmed to open and close each chamber at a specified interval, allowing users to perform
143 multiple, unattended incubations over the course of a single deployment. The chamber seals are
144 tapered to avoid the use of rubber O-rings that might have introduced a source of organic
145 contamination into the sample water. In experiments, the polycarbonate plastic used for the
146 PHORCYS incubation chambers reduced photosynthetically active radiation (PAR) within the
147 transparent chamber to 83% of incident strength; we present a means of accounting for this
148 attenuation, below. The opaque chamber was darkened by application of a coating to the outside of
149 the cylinder. All PHORCYS components are mounted to a stainless-steel frame, allowing the
150 instrument to operate to a depth of 100 m.

151 Dissolved oxygen concentrations in the two chambers are monitored with fast-response,
152 fluorescence quenching oxygen optodes (Aanderaa model 4531D; accuracy < 8 $\mu\text{M O}_2$; resolution <
153 1 $\mu\text{M O}_2$; response time < 30 s; Aanderaa Data Instruments, Inc., Bergen, Norway); each optode is
154 fitted into its chamber using a water- and gas-tight flange assembly. The instrument also has several
155 external sensors, including a third optode to monitor dissolved oxygen concentrations in the water
156 mass outside of the two chambers, PAR sensor, beam transmissometer, chlorophyll fluorometer, and
157 CTD. We did not investigate whether the arrangement of these sensors had any effect on the external
158 dissolved oxygen field surrounding the instrument.

159 With a full sensor suite, the nominal power consumption of the PHORCYS is 50 mA at 12V;
160 standby current is 2 mA. When programmed to sample at a 50 % duty cycle, a single 12V primary
161 'D' cell battery pack (20,000 mAh capacity) will power the instrument for up to 30 d in an

162 unattended deployment mode. Data are recorded in an ASCII fixed-field format onto a micro SD card
163 in a DOS-readable format. For attended deployments, a combination communications and external
164 power port provides the ability to observe data in real time, allow program updates, download data,
165 and power the instrument indefinitely. The sampling interval is nominally set to 1 min, though data
166 can be collected as frequently as every 15 s. The acquisition program determines sampling activity by
167 way of a real-time clock. The chambers can thus be programmed to open and close at any time,
168 allowing the investigator to make multiple incubations of any desired length. In the configuration
169 used to acquire the data presented here, (Fig. 2, □ symbol; e.g., Fig. 3) the chambers were
170 programmed to open at or around sunrise and the same operation was repeated at sunset, providing
171 two incubations in each 24 h period that aligned with the beginning and end of the photoperiod. The
172 chambers are opened and then closed sequentially (i.e., one after the other) to reduce total current
173 draw from the power source. The chambers remain open for 30 min at the outset of each incubation,
174 providing sufficient time for the water to be fully exchanged before closure; we confirmed this
175 flushing time was sufficient in both quiescent and flowing ($\sim 1 \text{ m s}^{-1}$) waters using a series of tests
176 with a tracer dye (results not shown). While we used a standard 30 min flush time in the deployments
177 for which data is presented below, any time can be specified in the instrument's control software,
178 which is written in BASIC.

179 The earlier PHORCYS data from 2012 (Fig. 2, ● symbols; e.g., Fig. 4) were obtained using a
180 prototype instrument that permitted only one incubation cycle per deployment. This prototype (Fig.
181 1b) was assembled from two 2.5 L Niskin-style sampling bottles mounted to an aluminum frame
182 (opaque polyvinylchloride and transparent polycarbonate plastic, respectively; actual usable volume,
183 2.6 L; General Oceanics, Inc., Miami, FL, USA). Closure of the chambers for incubation was
184 effected using an electrolytic time release (i.e., “burn wire”) system. Prior to deployment, the Niskin
185 bottle endcaps were cocked open and the retaining cable was rigged to a fusible burn wire plug. Once

186 in the water, a sufficient current was applied to the burn wire at a time set by the user, corroding the
187 wire and allowing the bottle endcaps to close. The chambers were then sealed and the incubation
188 began. For the deployments presented here, we programmed the chambers to close approximately 45
189 min after the PHORCYS had reached the desired depth. In the prototype instrument, Aanderaa model
190 4330F optodes (accuracy < 8 μM O_2 ; resolution < 1 μM O_2) were used to record dissolved oxygen
191 concentrations.

192 *Instrument deployments*

193 We conducted 6 unattended deployments of the PHORCYS in 3 distinct ecosystem types in
194 the North Atlantic basin (Fig. 2; Table 2; Supplemental Table 1). Open-ocean deployments (2 to 7 d
195 in length, using the prototype instrument) were conducted during cruises aboard the R/V *Knorr*;
196 during these deployments, the instrument was suspended at various depths in the euphotic zone from
197 a drifting surface buoy (Fig. 1c). Deployment and recovery were accomplished in 45-60 min from a
198 standard oceanographic research platform (Supplemental Fig. 1). An Argo satellite beacon (Fig. 1c)
199 allowed us to track the array remotely between deployment and recovery while the ship traveled up
200 to 300 km away to conduct other shipboard scientific operations; we specifically designed both
201 models of the PHORCYS to be wholly autonomous, incurring a minimal burden on other shipboard
202 operations. Pierside deployments were conducted using the present, multiple-incubation version of
203 the PHORCYS at the Iselin Marine Facility, Woods Hole, MA, USA (41° 31' 24" N 70° 40' 20" W);
204 the site adjoins a highly productive coastal embayment. In both cases, oxygen concentrations (μmol
205 L^{-1} O_2), percent saturation, and temperature were then recorded for each chamber at 1 min intervals.
206 Post-acquisition corrections for salinity were applied to both the open-ocean and coastal data using
207 concurrent observations of salinity and manufacturer-supplied correction coefficients. Concurrent
208 salinity data were obtained from the continuous, flow-through CTD system aboard the R/V *Knorr*
209 (for open-ocean deployments) or from the Seabird CTD unit mounted as an external sensor on the

210 present PHORCYS model (2016 coastal deployments). Due to the limited number of at-sea
211 deployments of the PHORCYS prototype and the challenges we encountered during our initial
212 cruises, we pooled our prototype results with the data we later obtained from the production-model
213 instrument in order to assemble a larger, more robust dataset for subsequent analysis.

214 *Instrument calibration and choice of deployment depth*

215 Optodes were calibrated before each pierside deployment and prior to each research cruise
216 using a two-point method, assuming a linear response between end-members. An air-saturated
217 solution was obtained according to manufacturer instructions by bubbling ambient air for approx. 30
218 min through a sufficient volume of Milli-Q water using an aquarium stone; a zero-oxygen solution
219 was obtained by dissolving an excess of reagent-grade sodium sulfite into a beaker containing Milli-
220 Q water. The optodes were then calibrated at atmospheric temperature and pressure, as recommended
221 by the manufacturer. At the open-ocean stations, we deployed the prototype instrument within the
222 euphotic zone at depths where the observed flux of photosynthetically active radiation (PAR;
223 wavelengths 400-700 nm) was between 10-30% of the incident flux at the surface. The depth of the
224 euphotic zone (z_{eu} , defined as the depth at which $PAR = 1\%$ of incident intensity) was identified
225 using profiles from shipboard hydrocasts. At each of these stations, we calculated an equivalent
226 deployment depth (z_{equiv}) that accounted for light attenuation by the transparent chamber's
227 polycarbonate plastic, according to a modification of the standard equation for the exponential decay
228 of light with depth:

$$229 \quad I_z(PAR) = \frac{I_0(PAR)e^{-K_d(PAR)z_{equiv}}}{T(PAR)} \quad (1)$$

230 where $I_0(PAR)$ and $I_z(PAR)$ are, respectively, the incident PAR intensity and intensity at depth z ,
231 $K_d(PAR)$ is the diffuse attenuation coefficient for the PAR spectral band (calculated from the

232 hydrocast profile at each station), and $T(PAR)$ is the transmissivity of the polycarbonate plastic
233 expressed as a fraction (0.83). Pierside deployments of the present PHORCYS model were conducted
234 at a depth of 1.5 m.

235 *Instrument validation by two independent methods*

236 First, to validate the optodes' ability to accurately track respiration, we used a standard
237 analytical method — two-point Winkler titration — to determine dissolved oxygen consumption in
238 triplicate water samples at the beginning and end of each incubation period (present model
239 instrument) or deployment (for data obtained with the prototype). Winkler titrations were conducted
240 in 125 mL BOD bottles according to EPA Method 360.2 as modified for shipboard determination in
241 seawater (Knapp et al., 1989). Initial Winkler titrations were made in samples collected within 15
242 min of deployment using a Niskin or Go-Flo bottle suspended at the same depth as the instrument. A
243 set of three darkened 125 mL BOD bottles containing water from the same Niskin or Go-Flo bottle
244 was incubated at *in situ* temperature until the PHORCYS was recovered or the incubation period
245 ended; these samples were then sacrificed according to the same protocol. The BOD bottles used in
246 these incubations were triple-rinsed with 10 % HCl and then Milli-Q water prior to sampling. All
247 reagents for Winkler titrations were A.C.S. grade or better; the sodium thiosulfate titrant was
248 standardized daily. Amperometric titration was performed using an autotitrator (Metrohm 904
249 Titrando; Metrohm USA, Inc., Riverview, FL).

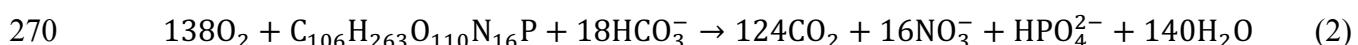
250 As an additional means of comparison during the open-ocean deployments, we also tracked
251 changes in dissolved oxygen in a series of continuously monitored shipboard bottle incubations.
252 Water from the PHORCYS deployment depth was retrieved for these incubations from a hydrocast
253 made within 1 h of deployment. Incubations were conducted in gas-tight, 300 mL glass BOD bottles;
254 at least 5 replicates were used for each series of measurements. Determination of dissolved oxygen

255 was made at 3 to 9 h intervals using optode spot minisensors (PreSens PSt3; response time < 40 s;
256 Precision Sensing GmbH, Regensburg, Germany; Warkentin et al., 2007) that were glued to the
257 inside surfaces of the bottles using food-quality silicone cement. The use of these optode spots
258 eliminated the need for drawing of aliquots from the sample bottles; the bottles had been soaked in
259 Milli-Q water for > 2 months following application of the sensor spots. Incubations were conducted
260 in the dark at *in situ* temperature as described in Edwards et al. (2011).

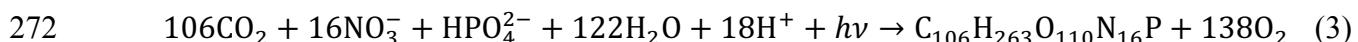
261 *Data analysis*

262 *PHORCYS rate calculations*

263 Volumetric rates of gross community respiration (GR) and net community production (NCP)
264 were calculated by linear least-squares regression of observations of dissolved oxygen concentration
265 over the length of the deployment (prototype model) or incubation period (present model). We
266 assumed the only significant chemical reactions contributing to oxygen consumption and evolution in
267 the two chambers were aerobic respiration and photosynthesis. For organic matter of elemental
268 stoichiometry roughly corresponding to that of Redfield (1934) and assuming production is based on
269 nitrate, these redox reactions can be represented as (after Stumm and Morgan, 2013):



271 and



273 As is the case with the classic light and dark bottle incubation technique, we assumed that only
274 respiration was taking place in the dark (i.e., GR; reported as positive quantities), while both
275 reactions were taking place simultaneously in the presence of light (i.e. NCP; in reality,

276 photorespiration also contributes to oxygen consumption in the light bottle). Finally, where possible,
277 we estimated the rate of gross primary productivity (GPP) according to the relationship

278
$$\text{GPP} = \text{NCP} + \text{GR} \quad (4)$$

279 To estimate uncertainties in PHORCYS rate estimates, we adapted an effective degrees of freedom
280 technique traditionally applied to time-series data in physical oceanography (Emery and Thomson,
281 2001). The method, which serves as an alternative to the standard error of the regression slope in
282 instances when true replication cannot be achieved, is described in detail in the Appendix.

283 *Rate calculations from Winkler titration samples and sensor spot incubations*

284 For the Winkler titration samples, GR was calculated using a difference of means,

285
$$\text{GR} = -\frac{d\text{O}_2}{dt} \quad (5)$$

286 where $d\text{O}_2$ is the difference between the mean dissolved oxygen concentration in samples sacrificed
287 at the final incubation timepoint and the mean measured at the initial timepoint and dt is the
288 incubation duration. For the sensor spot incubations, we calculated rates for each bottle using a linear
289 least-squares regression of all observations recorded over the time period; we then averaged the rates
290 obtained for the various replicates to obtain a final estimate. Uncertainties in rates based on the
291 Winkler titration method were determined from the standard deviations of the dissolved oxygen
292 concentrations measured in the replicates at each timepoint. For rates based on the sensor spot
293 incubations, we used the standard error of regression. While we report metabolic rate estimates from
294 the PHORCYS in oxygen-based units, a stoichiometric relationship such as the molar respiratory
295 quotient of Anderson and Sarmiento (1994) can be applied to convert these estimates to units of
296 carbon.

297 **Assessment**298 *PHORCYS metabolic rate estimates*

299 We observed a significant degree of daily and hourly variability in the time series data from
300 each PHORCYS deployment (e.g., Fig. 3 and 4). This variability manifested itself in a wide range of
301 metabolic rate estimates (Table 2; Supplemental Table 1) that reflect the interaction of multiple
302 biological and physical forcings, including diel changes in cellular growth cycle, surface-layer water
303 temperature, and irradiance. Daily rates of GR were estimated from dark chamber data for all
304 PHORCYS deployments (Table 2). For data obtained with the present model instrument, hourly rates
305 were calculated for each incubation segment (Fig. 3); these were extrapolated to daily rates to
306 facilitate comparison with other studies and with data from the prototype instrument. For open-ocean
307 data obtained with the PHORCYS prototype, daily rates were calculated using the entire DO time
308 series from each deployment. Erroneous readings from one of the optodes and a system malfunction
309 prevented us from recovering usable NCP data from the transparent chamber during two of the open-
310 ocean deployments of the prototype instrument. During the 24-27 April 2012 deployment, the chosen
311 depth of 29 m provided insufficient PAR (< 3% of surface intensity) to support measurable
312 photosynthesis in the transparent chamber; consequently, we could not calculate NCP or GPP for this
313 station (Supplemental Table 1). An obstruction prevented the transparent chamber from closing
314 during the November 2016 deployment, allowing us to recover useful data from only the dark
315 chamber.

316 We captured daily rates of GR ranging from $1.8 \pm 0.2 \mu\text{mol O}_2 \text{ L}^{-1} \text{ d}^{-1}$ at a mid-latitude
317 station in the North Atlantic to 10.5 ± 7.5 and $18.9 \pm 1.9 \mu\text{mol O}_2 \text{ L}^{-1} \text{ d}^{-1}$ in two different water
318 masses at the Woods Hole pier in early November (Fig. 3 and 4; Table 2). The wide variation in GR
319 we observed with the PHORCYS covers a significant range of the rates for marine systems compiled

320 by Robinson and Williams (2005). Daily rates of GPP at the PHORCYS deployment depth ranged
321 from essentially zero on several days at a mid-latitude station in the North Atlantic (Supplemental
322 Table 1) to $3.6 \pm 0.5 \text{ } \mu\text{mol O}_2 \text{ L}^{-1} \text{ d}^{-1}$ during a coccolithophore bloom (Collins et al., 2015) in the
323 sub-Arctic North Atlantic (Fig. 4). Rates of NCP at the deployment depth ranged from -2.0 ± 0.4
324 $\mu\text{mol O}_2 \text{ L}^{-1} \text{ d}^{-1}$ to $-4.2 \pm 0.2 \mu\text{mol O}_2 \text{ L}^{-1} \text{ d}^{-1}$ (Supplemental Table 1).

325 **Discussion**

326 *Subdaily variation in rates of metabolism; choices concerning data analysis*

327 Increasing evidence suggests the significant sub- and inter-daily variation in metabolic
328 activity captured by the PHORCYS exists in almost all natural aquatic systems (Caffrey, 2004;
329 Staehr et al., 2012; Collins et al., 2013); even in oligotrophic waters, respiration and production rates
330 may change significantly from one day (or hour) to the next, even as the system maintains an overall
331 state of near trophic balance (Aranguren-Gassis et al., 2012). The types of fluctuations we observed
332 in the various dissolved oxygen time series obtained from the PHORCYS appear to be characteristic
333 of incubation-based *in situ* instruments. McDonnell et al. (2015) and Boyd et al. (2015) both
334 observed similar patterns in dissolved oxygen data during recent deployments of an *in situ* device
335 that measures oxygen consumption rates on marine particles.

336 Nevertheless, the subtle changes in DO concentration we observed in multi-day incubations
337 with the PHORCYS prototype (e.g., Fig. 4) suggest that a single regression line — fitted, in this case,
338 to align with the closing and opening of the chambers — might have been poorly suited to a time
339 series exhibiting such variation. While one might instead have divided the full time series into shorter
340 segments to compute several different regressions (e.g, according to the photoperiod), we chose to
341 define the interval for rate calculations from the prototype instrument according the chamber opening
342 and closing times. This allowed us to avoid the bias inherent in dividing such a time series into

343 smaller segments. The present PHORCYS model allows users to predefine multiple, shorter
344 incubation periods of length appropriate to the ecosystem (e.g., Fig. 3); this feature eliminates the
345 need for a choice between either a subjective, *ex post facto* division of data or the application of a
346 single regression that might fail to capture the observed variation.

347 *Evaluation of instrument performance using independent methods*

348 PHORCYS estimates of community respiration were systematically higher than those
349 calculated for the same waters using the two-point Winkler titration method (Fig. 5a; Table 2). While
350 there was a significant correlation between estimates from the two different methods ($r^2 = 0.42$; $p =$
351 0.04), the traditional Winkler titration approach appeared to underestimate rates of respiration by
352 nearly one-third. Alternatively, the PHORCYS could have overestimated true rates of respiration
353 through artificial stimulation of the microbial community or inadvertent retention of residual
354 dissolved oxygen by the chambers or optode. In contrast, rate estimates from the PHORCYS
355 generally agreed with those based on our non-destructive optode sensor spot incubations, though we
356 had only five data points on which to evaluate the correlation (Fig. 5b; correlation was not
357 statistically significant).

358 Wikner et al. (2013) observed close agreement (~ 3 % deviation) between gross community
359 respiration rates derived from continuous optode measurements in a shipboard incubation chamber (1
360 L, clear glass) and a series of parallel incubations based on a Winkler method in 120 mL glass BOD
361 bottles. In previous work, we observed similar coherence between rates derived from a two-point
362 Winkler method in 300 mL glass BOD bottles and those based on incubations in 125 mL glass BOD
363 bottles fitted with optode sensor spots (surface area : volume ratios as reported in Table 1; Collins et
364 al., 2015). Underlying the agreement among methods in these previous studies is a common reliance
365 on incubations aboard ship. One might therefore conclude that the observed divergence between

366 estimates of respiration from the PHORCYS and those we made with the Winkler titration method
367 are due to inherent differences between the *in situ* PHORCYS approach and shipboard incubation
368 methods. However, evidence suggests that use of the same method is not even a guarantor of
369 agreement. For example, Robinson et al. (2009) found that vastly different rates were obtained from
370 the same method of measuring primary production when the only the timescale of incubation was
371 varied.

372 *Possible sources of observed discrepancies between methods*

373 *Gas permeability of materials*

374 The materials chosen for construction of the PHORCYS, particularly the polycarbonate
375 plastic we used for the incubation chambers, represent one possible source of bias in our method. We
376 chose polycarbonate material for its durability, low cost, and, compared with other plastics such as
377 polyvinylchloride, minimal biological reactivity. Polycarbonate was selected for its minimal
378 biological reactivity in construction of at least two similar *in situ* incubation devices (Langdon et al.,
379 1995; Robert, 2012). However, polycarbonate, like most plastics, is at least partially permeable to
380 dissolved oxygen; by comparison, the borosilicate glass of which BOD bottles are fabricated is
381 nearly impermeable (Kjeldsen, 1993; Robert, 2012). While some diffusion of dissolved oxygen
382 across the polycarbonate chamber walls could have occurred during incubations with the PHORCYS,
383 thus biasing our results, we believe the effect would likely have been very modest given the
384 dissolved oxygen gradients and timescales typical of our deployments. First, the gradient necessary
385 to drive any diffusion across the PHORCYS chamber wall (i.e., the difference between the internal
386 and external dissolved oxygen concentrations) did not exceed $25 \mu\text{mol O}_2 \text{ L}^{-1}$ during any
387 deployment; in the deployment presented in Fig. 3, the average difference between internal and
388 external concentrations was just $6.1 \mu\text{mol O}_2 \text{ L}^{-1}$. In a series of oxygen-diffusion experiments with a

389 device similar to the PHORCYS, Robert (2012) monitored the dissolved oxygen concentration inside
390 a polycarbonate chamber after the water inside was treated with sodium hydrosulfite to render it
391 anoxic. After manipulating the dissolved oxygen concentration in water outside the chamber to create
392 cross-boundary gradients ranging from approx. $50 \mu\text{mol O}_2 \text{ L}^{-1}$ to $> 350 \mu\text{mol O}_2 \text{ L}^{-1}$, the authors
393 observed little change in the dissolved oxygen concentration inside the chamber on timescales similar
394 to those of the PHORCYS deployments (11-12 h for the current version of the instrument; 3-5 d for
395 the PHORCYS prototype). Further, in a comprehensive study of the permeabilities of several gases
396 (O_2 , N_2 , CO_2 , and CH_4) in various polymers, Kjeldsen (1993) concluded that bias introduced by
397 permeability should be taken into account primarily when certain materials such as silicone rubber
398 were considered for use in anoxic waters; silicone rubber is more than 250 times as permeable to
399 dissolved oxygen than the polycarbonate plastic we used in construction of the PHORCYS (Kjeldsen,
400 1993).

401 *Handling or sampling bias*

402 Errors or bias introduced during the handling and manipulation of samples for bottle
403 incubations might also explain some of the discrepancy in rate measurements. For example, the
404 PHORCYS minimizes physical disturbances associated with seawater handling: Since the instrument
405 takes seawater samples and then incubates them in place, the planktonic community is not subjected
406 to rapid changes in temperature, pressure, and light associated with bringing water samples to the
407 surface via hydrocast and preparing them for shipboard incubations (Calvo-Díaz et al., 2011). The
408 PHORCYS also minimizes another potential bias that can arise when water containing marine
409 microbes is sampled from Niskin bottles; Suter et al. (2016) found that variation in settling rates
410 among marine particles can lead to an undersampling of microbial communities on faster-sinking
411 particles, which can fall below the bottles' spouts before aliquots can be drawn.

412 *Biofouling and surface colonization*

413 While we did not observe any significant biofouling of the PHORCYS or its components
414 during the deployments for which data are presented here, unwanted biological growth represents a
415 significant challenge and source of potential bias when autonomous sensors are deployed in the
416 surface and mesopelagic ocean (Delauney et al., 2010; Manov et al., 2004; e.g., Collins et al., 2013).
417 We imagine biofouling could represent a significant obstacle during future deployments if the
418 deployment duration were to exceed 5-7 d (the maximum explored in this work; Table 2), or if the
419 instrument were deployed in a productive ecosystem during a period of elevated primary production.
420 Compare, however, Robert (2012), who reported little biofilm growth on the chamber of a similar
421 incubation-based instrument after deploying it with no fouling controls for several months in the
422 Mediterranean Sea.

423 The lack of visual evidence of fouling in the PHORCYS chambers notwithstanding, there is
424 substantial evidence that microbial colonization of surfaces happens rapidly in the marine
425 environment (Salta et al., 2013; Dang and Lovell, 2016). It is therefore almost certain that at least
426 some microbial colonization of the chamber walls would have taken place within the 3-5 d timescale
427 of our deployments. Because we did not make measurements of biofilm activity or species
428 abundance, it is impossible to diagnose what contribution these communities might have made to the
429 observed fluxes in dissolved oxygen. However, compared to the surface area to volume ratios of the
430 150 and 300 mL vessels in which the other rate measurements were made (125 mL bottle, 0.83; 300
431 mL bottle, 0.77), the lower surface area to volume ratios of the PHORCYS chambers (current model,
432 0.34; prototype, 0.67) provide fewer opportunities for colonization relative to the volume being
433 incubated (Table 1). There is also some evidence that the small volumes typical of BOD bottles may
434 induce non-representative changes in the planktonic microbial community during incubations on
435 timescales of 24-48 h (Pratt and Berkson, 1959); however, compare Fogg and Calvario-Martinez

436 (1989), who found that such bottle size effects were only significant during periods of very high
437 primary productivity. A thorough, systematic assessment of these so-called wall and bottle effects
438 should be part of the community intercalibration we advocate in our conclusion.

439 *Other possible sources of bias*

440 Alternatively, the well-documented dependence of community respiration rates on
441 temperature (Yvon-Durocher et al., 2012) might explain the apparent disagreement between methods.
442 While temperatures within the PHORCYS chambers fluctuated only according to the movement of
443 tidal water masses (Fig. 3) or the natural warming and cooling of the surface layer (Fig. 4), the
444 temperature inside the incubator in which the Winkler samples were kept during the shipboard
445 deployments fluctuated during each series of experiments by $\pm 2^{\circ}\text{C}$ from the target. A further, more
446 intriguing explanation for the systematic discrepancy — pointing in this case to overestimation of
447 rates by the PHORCYS — is that dissolved oxygen could have accumulated on the optodes or inside
448 walls of the instrument chambers over the course of each deployment, in spite of the standard 30 min
449 flushing protocol. Wikner et al. (2013) offer convincing evidence for the accumulation of dissolved
450 oxygen on the optode surface and acrylic (polymethylmethacrylate) stopper used in their shipboard
451 incubation apparatus; the application of a correction factor for this bias reduced apparent rates
452 calculated from the optode time series relative to the Winkler method. We did not evaluate oxygen
453 retention by the polycarbonate plastic material of which the PHORCYS chambers were constructed.

454 The nature of linear regression itself may also play a role in differences observed between the
455 PHORCYS and the Winkler-based method. A least-squares regression line fit to a large set of
456 observations collected at high temporal frequency, such as those obtained from the PHORCYS, is
457 sensitive in some degree to each of those observations. In comparison, a rate calculated from the
458 beginning and ending oxygen concentrations in the two-point Winkler method is necessarily sensitive

459 only to those two observations; if some unrepresentative source of variability is present in just one
460 bottle, the entire measurement can be heavily biased. Regression of data from the PHORCYS thus
461 yields rate estimates which are robust to the sampling bias inherent in point measurements, yet the
462 technique leaves the underlying data intact to be further interrogated for information about natural
463 variability.

464 *The “deep breath” phenomenon*

465 Finally, while not evident in data from the deployments presented in Fig. 3 or 4, we did
466 occasionally observe a sharp initial decrease in dissolved oxygen concentration within the chambers
467 shortly after closure (data not shown). This phenomenon was reported extensively by Robert (2012)
468 during testing of a similar instrument in the Mediterranean Sea, and thus warrants further
469 investigation. The initial “deep breath” observed in these instances could reflect the rapid and
470 preferential utilization by the microbial community of a limited but highly labile fraction of the
471 dissolved organic carbon (DOC) pool within the chamber. Such a phenomenon has been observed
472 frequently in rates of DOC consumption in freshwater systems, where labile carbon is often
473 metabolized at a rapid rate in the initial minutes or hours of an incubation, leading to an apparent
474 decline in metabolic activity once the labile pool has been exhausted and the incubation progresses
475 (del Giorgio and Pace, 2008; Guillemette and del Giorgio, 2011; Guillemette, McCallister, et al.,
476 2013). While we did not directly observe a pronounced initial change in the rate of dissolved oxygen
477 consumption in any of our shipboard optode sensor spot incubations, it is possible that our sampling
478 interval (3 to 9 h; see above) was of insufficient resolution to detect it.

479 **Comments and recommendations**

480 Through autonomous collection of biogeochemical observations at uniquely high temporal
481 frequency, the PHORCYS yields estimates of community metabolic activity while simultaneously

482 freeing the analyst from the logistical constraints of attended water column sampling and preparation
483 of shipboard incubations. While we could not determine the origin of the systematic discrepancy
484 between the PHORCYS rate estimates and those based on the traditional two-point Winkler method,
485 the instrument's design allows investigators to avoid many of the potential biases associated with
486 bottle incubations that have been previously documented in the literature. The PHORCYS offers a
487 further advantage in that it can be used to collect information over multiple timescales about the
488 metabolic state of marine and aquatic ecosystems at minimal cost and burden to the user. While the
489 mixed-layer deployments we have presented here provided volumetric rates of ecosystem metabolism
490 at a single depth, multiple PHORCYS units could be deployed simultaneously at different depths in
491 the water column as a means of making depth-integrated rate measurements; one could compare
492 these rates to estimates obtained from *in situ* geochemical tracer studies. Future work could also
493 include direct, side-by-side comparison of metabolic rates obtained from the PHORCYS with those
494 from the classic, bottle-based ^{14}C or ^{13}C tracer methods.

495 In this spirit, we believe a new, community intercalibration effort is warranted to
496 systematically evaluate the many sources of uncertainty in incubation-based measurements of
497 community metabolism. The workshops of the Group for Aquatic Primary Productivity (GAP;
498 Figueroa et al., 2014) could serve as a model for such an effort, which should include comprehensive
499 evaluation of various combinations of incubation chamber materials, types of oxygen sensors,
500 chamber sizes, and incubation durations. Genetic and biogeochemical tools for characterizing the
501 extent, mechanisms, and effects of surface colonization within the incubation chambers would be
502 critical to the success of any such endeavor. We are optimistic such an effort might reveal the causes
503 of longstanding discrepancies such as those we observed between our PHORCYS rate estimates and
504 those we obtained with bottle-based methods.

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520 **Availability of data and code**

521 A MATLAB script to read in, process, and estimate rates and uncertainties in dissolved
522 oxygen data from the PHORCYS is provided online at
523 https://github.com/jamesrco/DO_Instruments/. The script can be easily adapted to calculate N_{eff} -
524 based estimates of uncertainty in any dissolved oxygen time series. All PHORCYS and Winkler
525 titration data and other scripts required to reproduce the results and figures in this work are available
526 online in the same location.

527

528 **Appendix**529 *A new method for calculation of uncertainties in metabolic rate estimates*

530 The ideal means of estimating uncertainties in PHORCYS rates would have been true
531 biological replication, i.e., the simultaneous deployment of several identical instruments in the same
532 water mass. One could then have used the standard deviation of the rate measurements in each
533 different instrument as an estimate of the overall uncertainty. Because we had only one instrument —
534 an exceedingly common situation in oceanographic work — such true replication was not possible.
535 The standard error of the regression slope provides one possible estimate of uncertainty in time-series
536 dissolved oxygen data; for example, this common approach was recently applied to data from *in situ*
537 chamber incubations of sinking marine particle material (McDonnell et al., 2015). However, we
538 assumed that the standard error of regression would significantly underestimate the true uncertainty
539 in our estimates since it does not take into account the reduced number of degrees of freedom in such a
540 time series. Because the data points in such a dissolved oxygen time series are not independent of one
541 another, there are almost always far fewer effective degrees of freedom N_{eff} in such data than the
542 number of observations (i.e., data points) N (Emery and Thomson, 2001). (We represent the effective
543 degrees of freedom by the notation N_{eff} in lieu of the N^* notation used by Emery and Thompson.)

544 Our approach was the following: For each time series of dissolved oxygen concentrations, we
545 first approximated the integral time scale T of the data according to

$$546 \quad T = \frac{1}{C(0)} \int_0^{B_0} C(\tau) d\tau \quad (A1)$$

547 where $C(0)$ is the value of the autocorrelation function C of the time series at lag $\tau = 0$, and B_0 is the
548 time lag of the autocorrelation function at the first zero crossing of the x -axis, which (after, e.g.,
549 Talley et al., 2011) we use as an estimate of the timescale of decorrelation. We then followed the

550 method of Emery and Thompson (2001) to estimate the effective number of degrees of freedom N_{eff}
551 from T , N , and Δt , where Δt is the sampling interval of the data:

552

$$N_{eff} = \frac{N\Delta t}{T} \quad (\text{A2})$$

553 In this formulation, $N\Delta t$ is therefore the total length of the oxygen time series in which the rate
554 estimate was made. Finally, we used this N_{eff} to obtain $s_{\hat{\beta}_{1,adj}}$, an adjusted estimate of the
555 standardized uncertainty in the slope parameter of the regression (i.e., $\hat{\beta}_1$, the rate of dissolved
556 oxygen consumption or production), according to

557

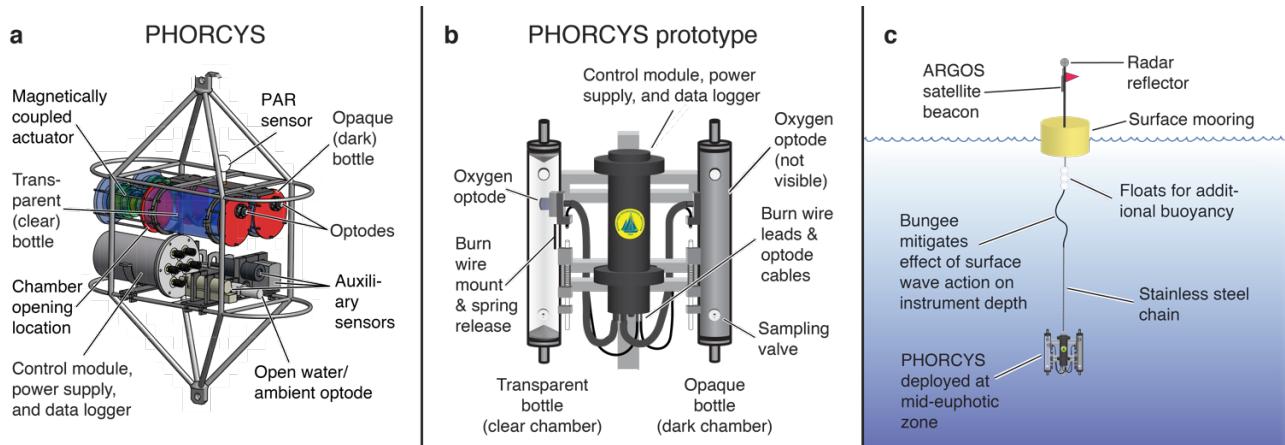
$$s_{\hat{\beta}_{1,adj}} = \sqrt{\frac{SSE}{N_{eff}-2} \frac{N}{\Delta}} \quad (\text{A3})$$

558 where SSE is the sum of the squared errors from the fit of the regression, i.e., $\sum_{i=1}^N (y_i - \hat{y}_i)^2$, N is
559 the number of observations (as above), and Δ is the determinant $NS_{xx} - S_x^2$. Eq. A3 is simply the
560 formula for calculation of the standard error of the regression slope in the unweighted case, except
561 that N_{eff} is used instead of N .

562 This method of estimating uncertainties in PHORCYS rates produced values of N_{eff} , the
563 number of effective degrees of freedom, which were typically $\ll N$, the number of observations in
564 the given dissolved oxygen time series (Table A1). Estimates of the integral time scale T ranged from
565 0.5 to 7.2 h; at station PS-2, the 77.4 h deployment for which data are presented in Fig. 4, we
566 estimated T to be 7.2 h (Table A1). Using the N_{eff} derived from these time scales, we obtained
567 adjusted uncertainty estimates for our PHORCYS rates ($s_{\hat{\beta}_{1,adj}}$) which were much greater in each
568 case than the standard error of the regression slope, $s_{\hat{\beta}_1}$ (compare mean precision of 24.8 % and 3.4
569 %, respectively; Table A1). While more robust than the corresponding $s_{\hat{\beta}_1}$, these $s_{\hat{\beta}_{1,adj}}$ still reflect a

570 fundamental limitation of linear regression: Both methods yield estimates of uncertainty which are
571 inversely proportional to the number of data points (i.e., the length of the underlying data series) and
572 the range of values spanned by the independent variable.

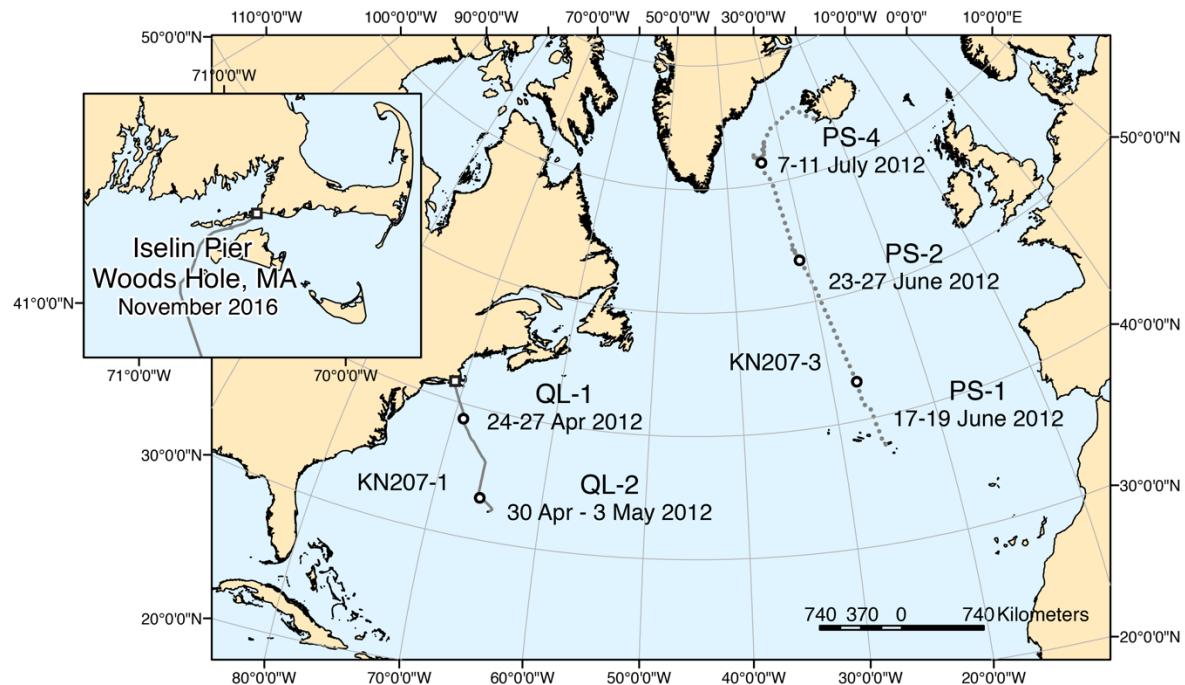
573 **Figures**



574

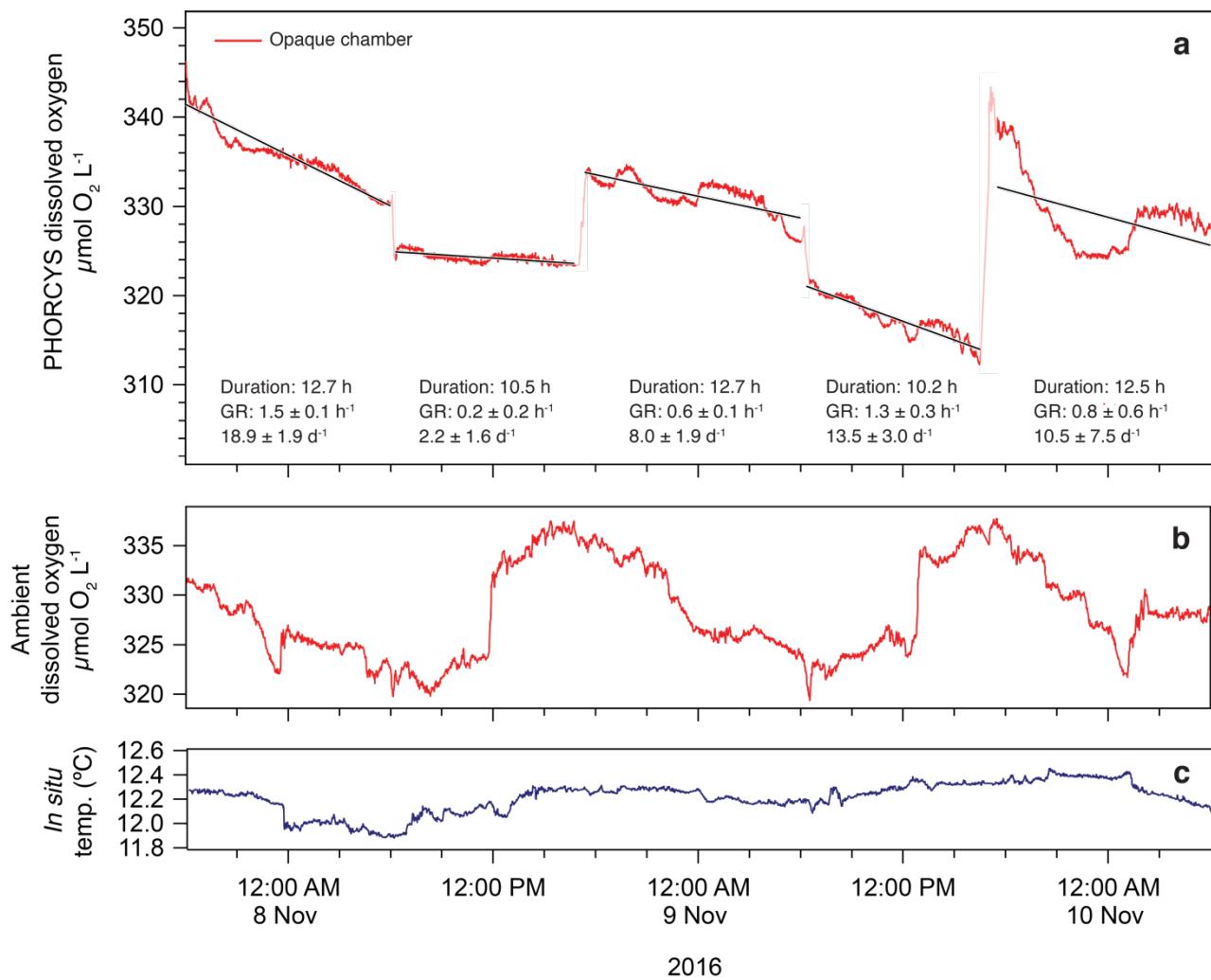
575 **Fig. 1.** Design and deployment of the PHORCYS. Major components of the current model

576 PHORCYS and prototype instrument are illustrated in (a) and (b), respectively. (a) The PHORCYS
 577 uses magnetically-coupled actuators, allowing for multiple openings and closings of the chambers
 578 during a single deployment. The current instrument also includes several sensors for collection of
 579 auxiliary data in the water outside of the chamber, including photosynthetically active radiation
 580 (PAR), conductivity, ambient temperature, transmissivity, and chlorophyll fluorescence. The
 581 PHORCYS also includes a third optode to measure dissolved oxygen concentrations in the ambient
 582 water mass outside of the two chambers. (c) Rigging scheme for open-ocean deployments from a
 583 drifting surface mooring, as described in the text.



584

585 **Fig. 2.** Locations of PHORCYS deployments described in the text. Primary map: Unattended open-
 586 ocean deployments from a surface mooring were conducted using the PHORCYS prototype at 5
 587 stations during two cruises aboard the *R/V Knorr*. Stations QL-1 and QL-2 were conducted during
 588 cruise KN207-1; PS-1, PS-2, and PS-3 were conducted during cruise KN207-3. Inset: Pierside
 589 deployments using the present PHORCYS model were conducted in November 2016 at the Iselin
 590 Marine Facility in Woods Hole, MA, USA.

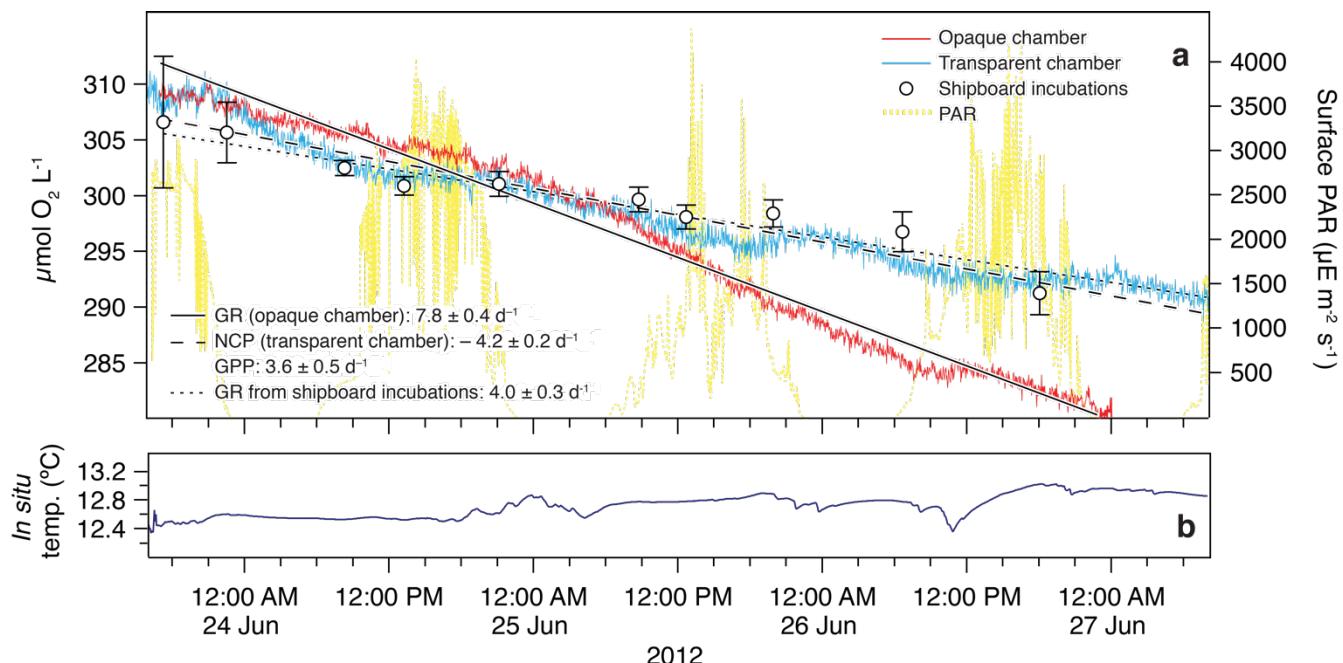


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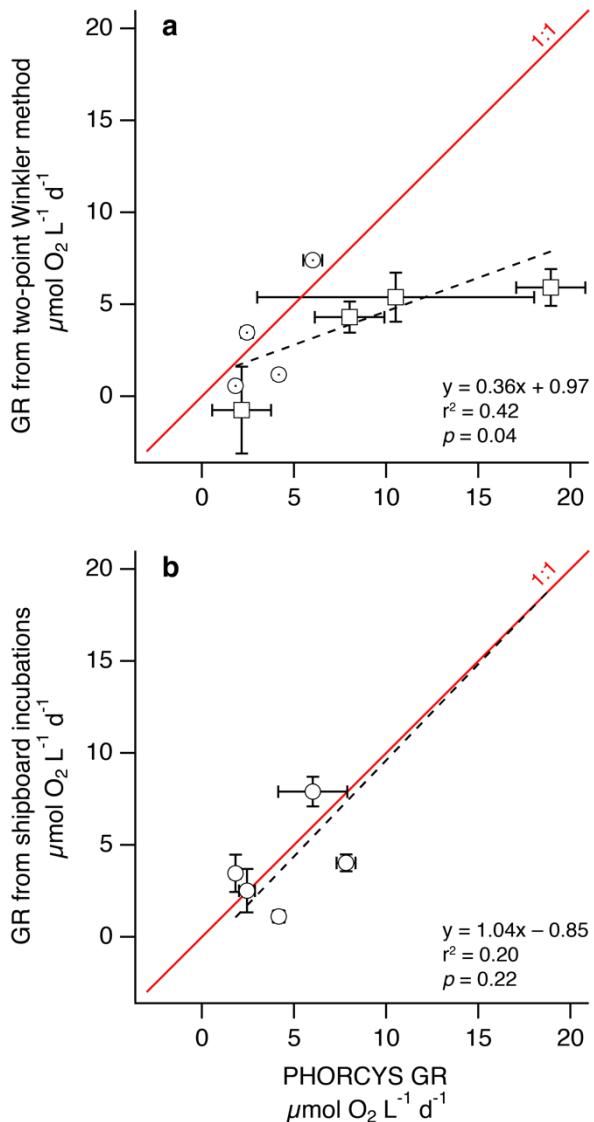
592 **Fig. 3.** Continuous, unattended observations of community respiration at the Iselin Pier in Woods
 593 Hole over a 3 d period in November 2016. (a) Record of dissolved oxygen concentration in the
 594 opaque PHORCYS chamber. The incubation periods from which estimates of GR were calculated are
 595 separated by 30 min, *in situ* flushing periods when the chamber was opened and closed to obtain a
 596 new water sample. Separate estimates of GR for each of the incubation periods (units of $\mu\text{mol O}_2 \text{ L}^{-1}$
 597 d^{-1}) were obtained by linear least-squares regression, and are shown as solid traces superimposed
 598 over the instrument data; these are the Iselin Pier data reported in Tables 2 and A1. Uncertainties
 599 were determined using the effective degrees of freedom method described in the Appendix. (b)
 600 Ambient *in situ* dissolved oxygen concentration measured concurrently outside the PHORCYS
 601 chambers, reflecting tidal changes in water-mass properties. (c) Ambient *in situ* temperature data

602 recorded outside the PHORCYS chambers. An instrument malfunction during the deployment
603 prevented us from recovering data from the transparent PHORCYS chamber.



604

605 **Fig. 4.** Unattended observations of ecosystem metabolism made with the prototype PHORCYS
 606 instrument at station PS-2 (Fig. 2; Supplemental Table 1) during a sub-Arctic, open-ocean
 607 deployment aboard the R/V *Knorr*. A midsummer bloom of a calcifying phytoplankton species was
 608 in progress at the site (Collins et al., 2015) when these observations were made. (a) Estimates (in
 609 units of $\mu\text{mol O}_2 \text{ L}^{-1} \text{ d}^{-1}$) of gross community respiration (GR) and net community production (NCP)
 610 were obtained by linear least-squares regression, and are shown as traces (GR as solid trace; NCP as
 611 dashed trace) superimposed over the instrument data. The prototype instrument allowed for only a
 612 single incubation over the course of the deployment. GPP was calculated as the difference between
 613 GR and NCP based on Eq. 4 in the text. Uncertainties were determined using the effective degrees of
 614 freedom method described in the Appendix. Incident photosynthetically active radiation (PAR) was
 615 measured using shipboard sensors. Dissolved oxygen concentrations measured concurrently in dark
 616 shipboard incubations using optode sensor spots (5 replicates; error bars show $\pm \text{SD}$) are
 617 superimposed as open circles. The respiration rate estimated from these incubations is shown as a
 618 dotted trace. (b) Diel warming of the surface layer is evident in *in situ* temperature data collected by
 619 the PHORCYS.



620

621 **Fig. 5.** Comparison of community respiration (GR) rate estimates from the PHORCYS (x-axis) with
 622 rates determined by (a) the two-point Winkler titration method and (b) a series of shipboard bottle
 623 incubations using optode sensor spots. Circles show data from the prototype instrument, while
 624 squares show data collected with the present PHORCYS model. A Type II (major axis) regression
 625 (dashed line) was fit to each set of paired observations using the lmodel2 package for R (Legendre,
 626 2014). In (a), the regression model was fit to a single dataset consisting of both current and prototype
 627 model data. A red 1:1 line is superimposed in each panel for reference.

628

629 **Table 1.** Estimated surface area to volume ratios of PHORCYS chambers and standard BOD bottles.
630

Bottle or chamber type	Actual usable volume (mL)	Estimated internal surface area (cm ²)	Estimated surface area : volume ratio
PHORCYS chamber (prototype)	2610	1760	0.67
PHORCYS chamber (current model)	5680	2035	0.34
Typical 125 mL BOD bottle	149.2 ± 0.3	124.4 ± 5.0	0.83
Typical 300 mL BOD bottle	299.2 ± 0.4	229.1 ± 4.3	0.77

631
632 The average volumes and surface areas reported in this table for BOD bottles were determined from
633 independent measurements of the dimensions of 10 different bottles of each size; these were chosen
634 at random from the Woods Hole Oceanographic Institution inventory.

635 **Table 2.** Rates of community respiration measured in opaque bottles using the PHORCYS and two independent, traditional methods.

Deployment dates	Incubation period	Incubation duration (h)	Location ^a	PHORCYS model ^b	Community respiration (GR) ($\mu\text{mol O}_2 \text{ L}^{-1} \text{ d}^{-1} \pm \text{uncertainty}$)		
					PHORCYS opaque bottle ^c	Shipboard incubations ^d	Two-point difference of Winkler titrations at $t=0$ and recovery ^e
24-27 Apr 2012	Entire deployment	71.6	QL-1	Prototype	1.8 \pm 0.2	3.2 \pm 0.7	0.6 \pm 0.1
30 Apr - 3 May 2012	Entire deployment	65.4	QL-2	Prototype	4.2 \pm 0.3	1.1 \pm 0.2	1.2 \pm 0.04
17-19 June 2012	Entire deployment	41.2	PS-1	Prototype	2.4 \pm 0.3	3.4 \pm 0.5	3.5 \pm 0.2
23-27 June 2012	Entire deployment	77.4	PS-2	Prototype	7.8 \pm 0.4	4.0 \pm 0.3	—
7-11 July 2012	Entire deployment	94.0	PS-4	Prototype	6.0 \pm 0.5	7.9 \pm 0.6	7.4 \pm 0.2
7-8 Nov 2016	17:15-06:00	12.7	Iselin Pier	Present model	18.9 \pm 1.9	—	5.9 \pm 1.0
8 Nov 2016	06:15-16:45	10.5	Iselin Pier	Present model	2.2 \pm 1.6	—	-0.8 \pm 2.4
8-9 Nov 2016	17:20-06:00	12.7	Iselin Pier	Present model	8.0 \pm 1.9	—	4.3 \pm 0.9
9-10 Nov 2016	17:30-06:00	12.5	Iselin Pier	Present model	10.5 \pm 7.5	—	5.4 \pm 1.3

636 ^a Cruise station or geographical location (Fig. 2); additional metadata for each station are provided in Supplemental Table 1637 ^a See Fig. 1638 ^c Uncertainty adjusted for effective degrees of freedom, as described in the Appendix639 ^d Mean of ≥ 5 replicates; uncertainty derived from standard error of regression slope640 ^e Mean of 3 replicates; uncertainty derived from standard error

642
643**Table A1.** Comparison of methods for estimation of uncertainties in dissolved oxygen time series data.

Deployment dates	Location ^a	PHORCYS community respiration (GR) ($\mu\text{mol O}_2$ $\text{L}^{-1} \text{d}^{-1}$)	No. obser- vations (N)	Incubation duration (h)	Est. integral time scale (h)	Effective degrees of freedom (N_{eff})	Estimated uncertainty ($\mu\text{mol O}_2 \text{ L}^{-1} \text{ d}^{-1}$)		Method precision (est. uncertainty as percent of rate measurement)	
							Standard error of regression slope ($s_{\hat{\beta}_1}$)	Adjusted estimate based on N_{eff}	$s_{\hat{\beta}_1}$	$s_{\hat{\beta}_{1,adj}}$
24-27 Apr 2012	QL-1	1.8	2150	71.6	1.1	66.5	0.03	0.18	1.6 %	9.9 %
30 Apr - 3 May 2012	QL-2	4.2	1964	65.4	3.1	21.0	0.03	0.28	0.7 %	6.7 %
17-19 June 2012	PS-1	2.4	1238	41.2	0.5	76.4	0.08	0.32	3.3 %	13.1 %
23-27 June 2012	PS-2	7.8	2323	77.4	7.2	10.7	0.03	0.43	0.4 %	5.5 %
7-11 July 2012	PS-4	6.0	2820	94.0	1.9	49.8	0.07	0.52	1.2 %	8.6 %
7-8 Nov 2016	Iselin Pier	18.9	765	12.7	1.3	19.8	0.29	1.87	1.5 %	9.9 %
8 Nov 2016	Iselin Pier	2.2	627	10.5	1.2	17.0	0.25	1.60	11.6 %	74.4 %
8-9 Nov 2016	Iselin Pier	8.0	760	12.7	1.6	16.2	0.26	1.89	3.2 %	23.6 %
9-10 Nov 2016	Iselin Pier	10.5	750	12.5	2.9	8.7	0.71	7.51	6.7 %	71.4 %
Mean					2.3				3.4 %	24.8 %

644

645

646

647

648

^a Cruise station or geographical location (Fig. 2); additional metadata for each station are provided in Supplemental Table 1

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