

1   **Authors:** James R. Collins<sup>1,2\*†</sup>, Paul D. Fucile<sup>3</sup>, Glenn McDonald<sup>4</sup>, Justin E. Ossolinski<sup>2</sup>, Richard G.  
2   Keil<sup>5</sup>, James R. Valdes<sup>3</sup>, Scott C. Doney<sup>2</sup>, and Benjamin A. S. Van Mooy<sup>2\*</sup>

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

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

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

9   <sup>3</sup> Department of Physical Oceanography, Woods Hole Oceanographic Institution, Woods Hole, MA  
10   02543, USA

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

13   <sup>5</sup> 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

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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   (*PHO*tosynthesis and *Respiration* Comparison-Yielding System) 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 (Reinthal 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  $^{14}\text{C}$   
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  $^{18}\text{O}$  or  $\text{CO}_2$  (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  $^{14}\text{C}$ -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

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

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

## **Materials and procedures**


### *Instrument design and operation*


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

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

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

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

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

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



in the water, a sufficient current was applied to the burn wire at a time set by the user, corroding the wire and allowing the bottle endcaps to close. The chambers were then sealed and the incubation began. For the deployments presented here, we programmed the chambers to close approximately 45 min after the PHORCYS had reached the desired depth. In the prototype instrument, Aanderaa model 4330F optodes (accuracy < 8  $\mu\text{M O}_2$ ; resolution < 1  $\mu\text{M O}_2$ ) were used to record dissolved oxygen concentrations.

### *Instrument deployments*

We conducted 6 unattended deployments of the PHORCYS in 3 distinct ecosystem types in the North Atlantic basin (Fig. 2; Table 2; Supplemental Table 1). Open-ocean deployments (2 to 7 d in length, using the prototype instrument) were conducted during cruises aboard the R/V *Knorr*; during these deployments, the instrument was suspended at various depths in the euphotic zone from a drifting surface buoy (Fig. 1c). Deployment and recovery were accomplished in 45-60 min from a standard oceanographic research platform (Supplemental Fig. 1). An Argo satellite beacon (Fig. 1c) allowed us to track the array remotely between deployment and recovery while the ship traveled up to 300 km away to conduct other shipboard scientific operations; we specifically designed both models of the PHORCYS to be wholly autonomous, incurring a minimal burden on other shipboard operations. Pierside deployments were conducted using the present, multiple-incubation version of the PHORCYS at the Iselin Marine Facility, Woods Hole, MA, USA (41° 31' 24" N 70° 40' 20" W); the site adjoins a highly productive coastal embayment. In both cases, oxygen concentrations ( $\mu\text{mol L}^{-1} \text{O}_2$ ), percent saturation, and temperature were then recorded for each chamber at 1 min intervals. Post-acquisition corrections for salinity were applied to both the open-ocean and coastal data using concurrent observations of salinity and manufacturer-supplied correction coefficients. Concurrent salinity data were obtained from the continuous, flow-through CTD system aboard the R/V *Knorr* (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

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

*Instrument validation by two independent methods*

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

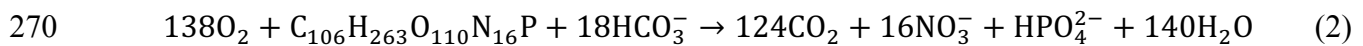
As an additional means of comparison during the open-ocean deployments, we also tracked changes in dissolved oxygen in a series of continuously monitored shipboard bottle incubations. Water from the PHORCYS deployment depth was retrieved for these incubations from a hydrocast made within 1 h of deployment.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

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

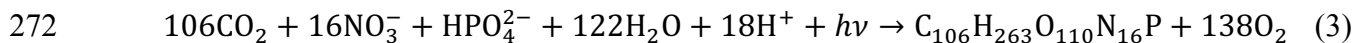
## Data analysis

### PHORCYS rate calculations

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



and



As is the case with the classic light and dark bottle incubation technique, we assumed that only respiration was taking place in the dark (i.e., GR; reported as positive quantities), while both 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  $10.5 \pm 7.5$  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

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

## Discussion

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

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

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

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

#### *Evaluation of instrument performance using independent methods*

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

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



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

#### *Possible sources of observed discrepancies between methods*

##### *Gas permeability of materials*

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

device similar to the PHORCYS, Robert (2012) monitored the dissolved oxygen concentration inside a polycarbonate chamber after the water inside was treated with sodium hydrosulfite to render it anoxic. After manipulating the dissolved oxygen concentration in water outside the chamber to create 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 observed little change in the dissolved oxygen concentration inside the chamber on timescales similar to those of the PHORCYS deployments (11-12 h for the current version of the instrument; 3-5 d for the PHORCYS prototype). Further, in a comprehensive study of the permeabilities of several gases ( $\text{O}_2$ ,  $\text{N}_2$ ,  $\text{CO}_2$ , and  $\text{CH}_4$ ) in various polymers, Kjeldsen (1993) concluded that bias introduced by permeability should be taken into account primarily when certain materials such as silicone rubber were considered for use in anoxic waters; silicone rubber is more than 250 times as permeable to dissolved oxygen than the polycarbonate plastic we used in construction of the PHORCYS (Kjeldsen, 1993).

#### *Handling or sampling bias*

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

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

#### *Other possible sources of bias*

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

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

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

#### *The “deep breath” phenomenon*

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

#### **Comments and recommendations**

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

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

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

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#### **Availability of data and code**

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

## 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 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



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

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

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

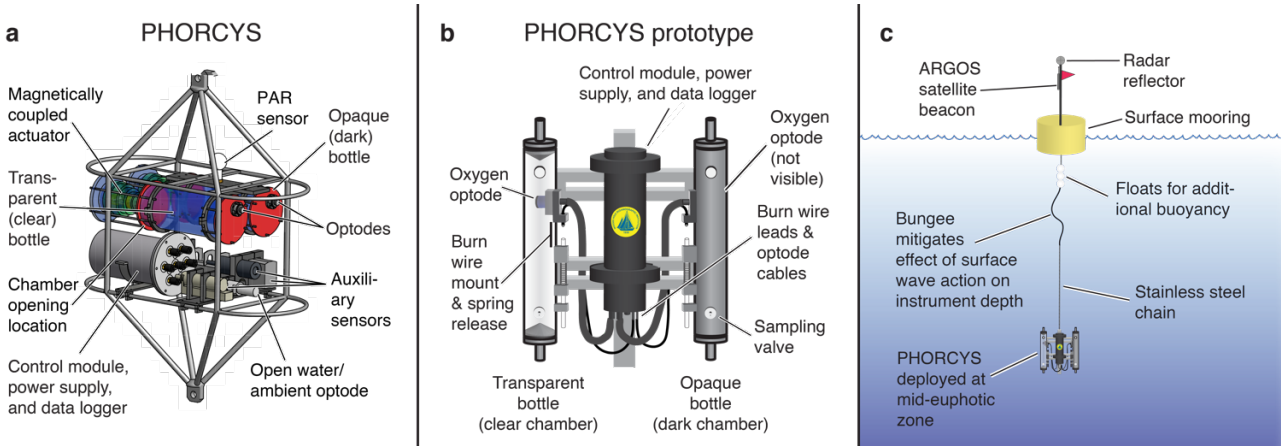
$$s_{\hat{\beta}_{1,adj}} = \sqrt{\frac{SSE}{N_{eff}-2} \frac{N}{\Delta}} \quad (A3)$$

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 the number of observations (as above), and  $\Delta$  is the determinant  $NS_{xx} - S_x^2$ . Eq. A3 is simply the formula for calculation of the standard error of the regression slope in the unweighted case, except that  $N_{eff}$  is used instead of  $N$ .

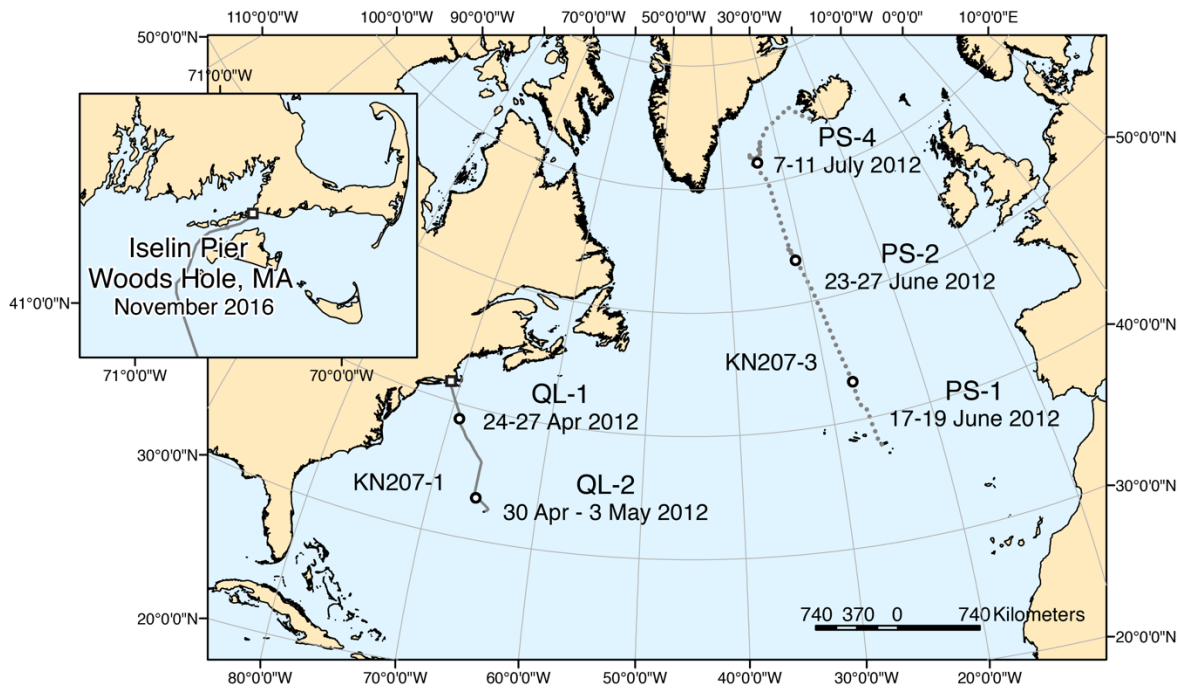
This method of estimating uncertainties in PHORCYS rates produced values of  $N_{eff}$ , the number of effective degrees of freedom, which were typically  $\ll N$ , the number of observations in the given dissolved oxygen time series (Table A1). Estimates of the integral time scale  $T$  ranged from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

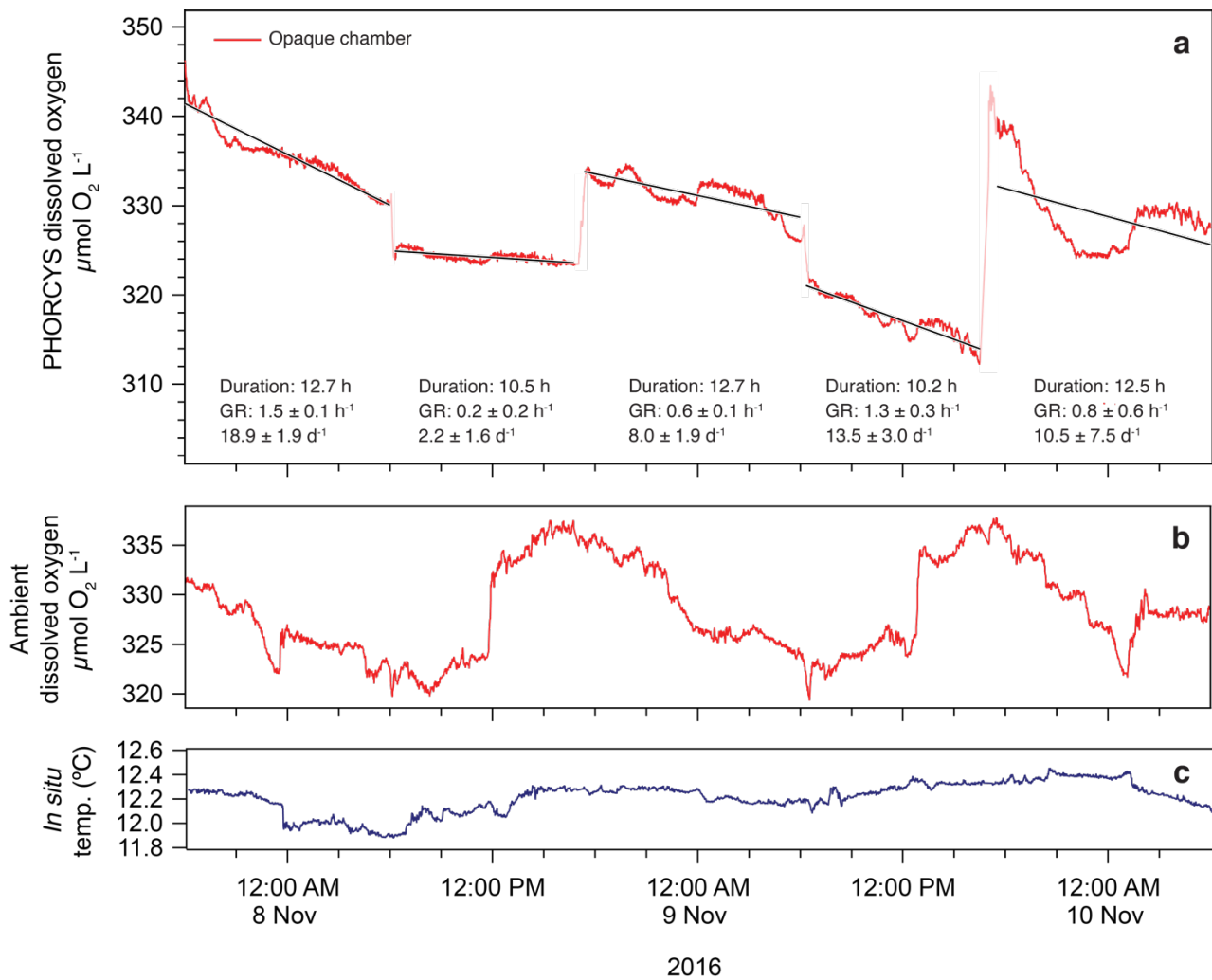
574



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.

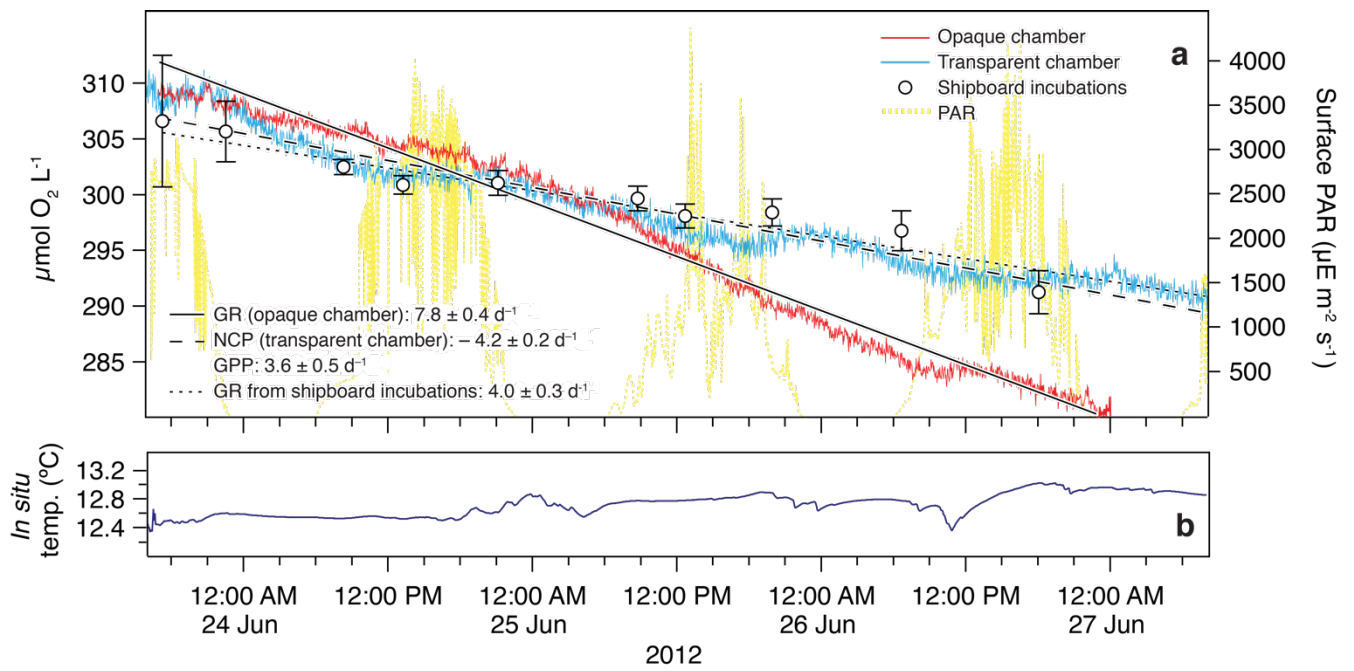


**Fig. 2.** Locations of PHORCYS deployments described in the text. Primary map: Unattended open-ocean deployments from a surface mooring were conducted using the PHORCYS prototype at 5 stations during two cruises aboard the *R/V Knorr*. Stations QL-1 and QL-2 were conducted during cruise KN207-1; PS-1,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

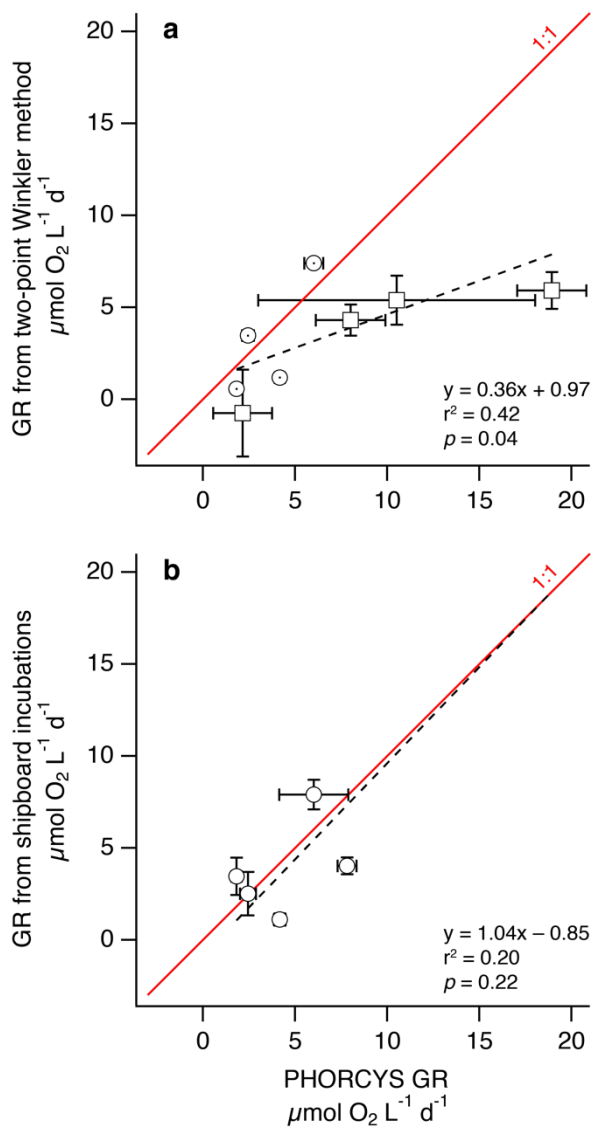


**Fig. 3.** Continuous, unattended observations of community respiration at the Iselin Pier in Woods Hole over a 3 d period in November 2016. (a) Record of dissolved oxygen concentration in the opaque PHORCYS chamber. The incubation periods from which estimates of GR were calculated are separated by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



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



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



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 <sup>2</sup> )	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 <sup>a</sup>	PHORCYS model <sup>b</sup>	Community respiration (GR) ( $\mu\text{mol O}_2 \text{ L}^{-1} \text{ d}^{-1} \pm \text{uncertainty}$ )		
					PHORCYS opaque bottle <sup>c</sup>	Shipboard incubations <sup>d</sup>	Two-point difference of Winkler titrations at $t=0$ and recovery <sup>e</sup>
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  
637 <sup>a</sup> Cruise station or geographical location (Fig. 2); additional metadata for each station are provided in Supplemental Table 1  
638 <sup>a</sup> See Fig. 1  
639 <sup>c</sup> Uncertainty adjusted for effective degrees of freedom, as described in the Appendix  
640 <sup>d</sup> Mean of  $\geq 5$  replicates; uncertainty derived from standard error of regression slope  
641 <sup>e</sup> Mean of 3 replicates; uncertainty derived from standard error

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

Deployment dates	Location <sup>a</sup>	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 $s_{\hat{\beta}_{1,adj}}$ 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 %

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

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