An in situ incubation method for measuring the productivity and responses of under-ice algae to ocean acidification and warming in polar marine habitats

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Abstract

During the Antarctic spring, algae grows under extensive areas of sea-ice and is a fundamental source of primary production. Understanding how under-ice (bottom-ice) algae will be affected by ocean warming and acidification is critically important in determining the probable future flow-on effects to the ecological communities this algae supports. To investigate this we designed and built a customised experimental system to assess the in situ responses of under-ice algae to changes in both seawater pH and temperature. We conducted two trials in 2013 followed by a successful 14-day incubation experiment in 2014 in the Ross Sea, Antarctica, using the system described here. Assessment of our main control parameters indicated we could reliably control and monitor both pH and temperature in transparent under-ice chambers. The “plug-and-play” nature of our novel system meant it was easy for divers to deploy and maintain in the very cold temperatures experienced under the sea-ice. Moreover, the system could be remotely sampled from a surface laboratory. This enabled robust monitoring and analyses of manipulated seawater conditions (e.g., pH and temperature), and of responses of the associated biological communities (e.g., fluxes in dissolved oxygen and nutrient levels).

Spring in Antarctica is a time of extensive sea-ice formation covering some 20-million square kilometres of coastal oceans. With long periods of daylight penetrating through the sea-ice, it is also a time of high under-ice (bottom-ice) algal productivity. Under-ice microalgae, generally dominated by diatoms (see McMinn et al. 2010a), can reach extremely high densities on the under-surface of sea ice (Arrigo 2014). At these high densities they provide a vital source of primary production that supports benthic and pelagic biodiversity and underpins food webs (Dayton and Oliver 1977; Thomas and Dieckmann 2002; Thrush et al. 2006; Norkko et al. 2007; Wing et al. 2012). These microalgae grow in a unique environment, with light transmitted through thick sea ice above, and a crystalline matrix of ice providing highly structured attachment substrate (Thomas and Dieckmann 2002). Understanding how ice algal based communities might be affected by ocean warming and acidification, both of which are major threats facing polar marine environments

Aronson et al. (2011; Smith et al. 2012; Schmidtko et al. 2014) is important in predicting future responses of these ecosystems to change. Antarctic primary producers are predicted to benefit from warmer seawater and elevated CO2 (the latter because of more carbon available for fixation) (Riebesell et al. 2007; Arrigo et al. 2012). The magnitude of the potential increases in primary production, and the associated changes in nutrient uptake and cycling, are all key to understanding flow on effects to higher consumers. Investigation of the influences of predicted climate related changes on under-ice algal productivity requires experimentally manipulated, and appropriately replicated (Cornwall and Hurd, 2015), treatments of differing pCO2 concentrations and/or temperatures that can be maintained for sufficiently long periods of time to elicit biological responses. However, the complex nature both of the sea ice habitat and the multi-species communities it supports makes it both impossible to recreate an ice-associated ecosystem in a laboratory setting, and logistically extremely challenging to study in situ. In the latter case, such experiments would require some form of under-ice diving support in extremely cold seawater. In addition, there are obviously various technical challenges involved in keeping scientific equipment operating in the
extreme cold and submerged conditions. As a result of these difficulties, to date, there have been very few in situ measurements of under ice algal productivity, and none that have simultaneously examined photosynthetic production and nutrient uptake. To address this challenge we designed and constructed an integrated manipulation and sampling system which was then successfully deployed to address these questions in intact sea ice ecosystems at two high latitude ice covered coastal sites in the Ross Sea, Antarctica.

We considered several approaches for estimating levels of primary production in our experimental system. One was to measure fluxes in a sealed (closed-system) incubation chamber by examining changes in water chemistry over time (see Tenburg et al. 1995). Such incubations are the traditional and often preferred methods for measuring primary productivity in situ (e.g., light and dark bottle incubations used to measure oxygen evolution, C\textsubscript{13} incorporation, dissolved inorganic carbon (DIC) removal in limnological and oceanographic research). Similarly, research on benthic communities may use chambers that enclose both the underlying substrate and overlying water to assess areal rates of production and uptake by quantifying temporal changes in the overlying water chemistry. However, these limit the experimental period to relatively short time frames, as biologically-mediated utilisation of solutes and build-up of wastes will eventually begin to alter experimental conditions and introduce artefacts. Other techniques that do not necessarily involve incubations in chambers include proxy measures for evaluating primary production, such as pulse amplitude modulated fluorometry (PAM) (e.g., Gradinger, 2009; McMinn et al. 2010\textit{a}; Napoléon and Claquin, 2012) and eddy covariance techniques (Berg et al. 2003, 2013). Both these methods have been used to assess productivity in algal dominated under-ice habitats (McMinn et al. 2010\textit{b}; Else et al. 2015). However, neither PAM nor the eddy covariance methods may necessarily lend themselves to the simultaneous quantification of photosynthesis and inorganic nutrient uptake.

In principal the issues identified above can be overcome with the use of flow-through incubation systems, where water of a relatively constant composition is delivered to chambers at a constant rate for an indefinite period of time (Miller-Way and Twilley 1996). In ocean acidification research such flow-through systems are commonly used for examining biological responses to changes in p\textsubscript{CO}_2 concentrations and are referred to as Free-Ocean \textsubscript{CO}_2 Enrichment (FOCE) experiments (see review by Gattuso et al. 2014). FOCE systems are open or semi-enclosed, typically 0.25–2 m\textsuperscript{2} in area, and 0.06–2 m\textsuperscript{3} in volume (Gattuso et al. 2014). They are preferable to completely closed systems, as they enable some percentage of the seawater in the enclosure to be replaced at a defined rate with external (new) water. This reduces issues such as limitation of nutrients as a consequence of biological activity in closed systems, which ultimately limits the duration of experiments. In these open or semi-open enclosures, certain chemico-physical factors (e.g., p\textsubscript{CO}_2, pH, or temperature) can be manipulated for prolonged periods in situ while the other components of the ecosystem remain at otherwise near natural states.

Thus, for assessing rates of primary production and nutrient uptake in under-ice habitats, we designed a system of fit-for-purpose incubation chambers, and their associated control and sampling equipment. Some of the challenges specific to this cold water, under ice environment that we needed to overcome included: (1) Gravity. We needed a way to keep incubation chambers firmly attached to the under surface of the ice; (2) Hard ice. Unlike benthic experiments, where chamber edges can be pressed into the sediment to seal from overlying waters, the hard under-surface of the ice presented difficulties in achieving a water-tight seal; (3) Bubbles. SCUBA divers were required to place the equipment in position, but their exhalent bubbles can also disturb the under-ice algal habitat; (4) Equipment failures. Quick and easy plug-and-play replacement of components underwater was necessary in the event of failure; (5) Slow rates. Fluxes of dissolved oxygen and inorganic nutrients are generally limited by the extremely cold seawater temperatures of the high Antarctic (Lohrer et al. 2013). Thus we needed to maximise our chances of detecting subtle changes in solute concentrations by facilitating easy, remote and frequent sampling; (6) Seawater manipulations. To assess the effects of ocean acidification and warming on under-ice algal productivity, maintenance of distinct pH treatments in situ for ecologically meaningful periods of time was critical. Moreover, as pH both affects and is affected by biological processes such as photosynthesis and respiration, we needed a means of controlling pH while also measuring how it changed after interacting with the enclosed biology; (7) Good replication. Quantifying the variation of responses was of key importance in understanding the real differences between treatments, and therefore in making meaningful ecological inferences.

Our methods involved: (1) specialized chambers for deployment in situ; (2) an above-ice portable laboratory system for the precise control of seawater characteristics; and (3) a seawater delivery and sampling system running between the chambers and the above-ice control system. Each component of the system was custom designed for maximum effectiveness in achieving our research aims in an extremely cold and challenging environment, where robustness, redundancy, and real time monitoring of experimental systems were all crucial for success.

**Materials and procedures**

**Under-ice chambers**

Sixteen identical chambers were constructed as shown in Fig. 1. This number of chambers was chosen to allow up to four treatment levels with four replicates each. As the
primary purpose of our chambers was to study the productivity of under-ice algae, they were constructed from strong highly transparent materials to allow maximum light transmission, and that could withstand cold conditions without breakage. Each chamber was constructed from a sheet of clear 2 mm thick polycarbonate plastic measuring 60 cm high × 215 cm long. A large cylinder (68 cm diameter; Fig. 1) was formed by joining the 60 cm edges of each sheet with a 3 cm overlap, which was then riveted, glued and also sealed together (Weldon 16). The cylinders were open at the top so that the area of under-ice algal habitat enclosed by each chamber was 0.36 m². Inverted cones of the same polycarbonate material were fitted, welded and glued inside each cylinder to form the conical chamber base (Fig. 1).

To hold the chamber up against the underside of the ice, we created a space that could be filled with air to create buoyant lift (Fig. 1B). This space was filled with an air volume of approximately 20 L (i.e., displacing 20 L of water thereby giving approximately 20 kg of lift). Installation of the chambers involved divers approaching the deployment area, then positioning themselves underneath the chamber so that their exhaled bubbles were captured in the buoyancy compartment (thus avoiding contamination of the sea ice within the chamber). The upward buoyant force of the trapped air held the chambers firmly in place without the need for ice screws or any other type of anchoring (see Plate 1). The upper rim of the chamber was stiffened with a 50 mm wide strip of clear 5 mm polycarbonate, and a silicon door-seal gasket was clipped over the top edge (Fig. 1B). The silicon gasket had a hollow bead section that conformed to the contours of the ice’s under-surface when compressed by buoyant lift, forming a mechanical seal between chamber edge and the ice under-surface.

The water volume enclosed by the chambers was 144 L, and the seawater supply rate that we used was 200 mL min⁻¹, therefore resulting in a water residence time inside each chamber of ~12 h. This residence time was selected to maximise our ability to detect potentially small rates of change in solute concentrations (expected in the extremely cold seawater). The relatively low seawater supply rate, however, would have been insufficient to keep the chamber waters well mixed. Water mixing is critical for avoiding biases in flux calculations, and was especially important in our application, given that the volume of the chamber was so much larger than the volume of our water samples (60 mL), and that temperature manipulation treatments would have become stratified. The mechanisms for seawater supply, sampling, and pulsed non-directional stirring are detailed in the sections below.

The water contained in each chamber was able to be sampled repeatedly and remotely during our trials and main experiment (to assess changes in dissolved oxygen, inorganic...
nutrients, pH, and a number of other parameters). The conical bottom of the chamber (with a collection jar positioned at the apex) was designed to capture algal material settling down from the ice surface area above. The attached under-ice algae was sampled at the end of the incubation, immediately following chamber removal.

To evaluate if there was any effect of the chambers themselves on the light levels within them, a Biospherical® scalar (QSPL-2200 Laboratory sensor) PAR (Photosynthetic Active Radiation) probe was used to make pairwise measurements inside and outside of chambers under the ice. In addition, in our main experiment in 2014, PAR loggers (Odyssey Data-Flow®; see Long et al. 2012 for an evaluation of these instruments) were deployed inside each chamber to measure the down-welling light in each chamber. A combination of Licor and Odyssey PAR loggers were also deployed to measure ambient light outside of the chambers and also above the ice to measure surface light.

**Portable laboratory system**

An automated system for controlling seawater supply flow rate, at a defined pH and temperature to the under-ice chambers, was established inside a tent on the surface of the sea ice. The seawater pH control component was designed in accordance with published recommendations (see Riebesell et al. 2010). This system consisted of three main integrated components: (1) a main control panel that housed pH and temperature controllers, low voltage isolating transformers, pinch valves and various other electrical components; (2) a set of insulated plastic “header tanks” (55 L) for controlling the pH required by the different seawater treatments; and (3) two 8-channel peristaltic pumps for delivery of seawater from header tanks, through small diameter (3.75 mm) Teflon tubing, to the individual incubation chambers deployed on the under-surface of the ice up to 20 m away. Ambient seawater was pumped from ~2 m beneath the ice and distributed evenly through a common lagged supply manifold into four pH control header tanks (Fig. 2). Supply (flow) of seawater to each header tank was demand-regulated using a small low pressure ballcock inlet valve located in each tank. The insulation of the header tanks, the even distribution of ambient seawater to them through the lagged manifold, and their positioning at floor level in the field tent acted to keep tank water temperatures close to ambient (−1.6°C).

**pH control**

For control and real-time monitoring of seawater pH, each header tank had a pH probe (Sensorex S150C) and an independent temperature probe (PT100) for automatic temperature compensation. Note electronic probe pH values were measured on the total hydrogen scale. Calibration of pH probes was conducted with a three-point curve using standard HACH laboratory buffers for pH 4.01, 7.00, and 10.01. Tris-hydroxymethyl aminomethane (TRIS) and 2-amino-2-methyl-1-propanol (AMP) buffers were then used to calculate the control pH offset (see later in the Assessment section and also Fig. 6). The pH and temperature analogue inputs were connected to an Omega pH controller (PHCN-
Control pH in each header tank was achieved by semi-continuously dosing food grade CO₂ to a diffuser coil made from a short length of thin-walled silicon tube (3 mm I.D., 0.5 mm wall thickness). The diffuser coil was positioned inside one end of a 400 mm section of 80 mm waste pipe with a HX-6830 Hailea pump mounted at the opposite end. The outflow of the pump was directed toward the diffuser coil to improve mixing of diffused CO₂. The supply of CO₂ to each silicon diffusion coil was controlled by a two-way 24 Vdc pinch valve (BioChem Fluidics, 100P3MP24-02S) located in the main control panel. Regulation of pH in each header tank was achieved by a simple feedback loop using the Omega controller to switch the power applied to the pinch valve, thus reciprocally pressurising and depressurising the CO₂ diffusions coils.

Temperature control

Temperature control was achieved in situ inside the individual chambers (rather than in header tanks) with 300 W low-voltage (24 Vac) pocket heating elements. This was considered the only practical way to control temperature since any heating done in header tanks, for example, would have completely dissipated to the surrounding water in transit along the 20 m umbilical cable. Each heating element was housed in the chamber control unit (CCU; described below) at the end of the umbilical cable, and was connected via a cable to an individual Omega® CN742 temperature controller and its “slave” solid state relay located in the portable laboratory system control panel. The temperature of the chamber seawater was monitored in real time via the display of the CN742 controller connected to a PT100 4-wire temperature probe located in the CCU. A line insulation monitoring device (Bender® IR423) was used for monitoring the electrical integrity of the underwater low voltage power supply as an additional safety measure.

Seawater delivery and sampling system

Seawater delivery

A pump located in each header tank supplied seawater (HX-6830 Hailea®), at a positive pressure to minimise seawater out-gassing, to one of two peristaltic pumps (MasterFlex® L/S 6-600 drive coupled to 8-channel heads and L/S size 24 silicon tube bridges). These pumps then delivered the seawater through Teflon tubes incorporated in umbilical cables (see next section) to each under-ice chamber at a controlled flow rate (in our case, 200 mL min⁻¹). Flow indicators (Scienceware Roto-Flo®) were positioned in series within each channel just after the peristaltic pump for visual verification that treated seawater was being delivered to each chamber at appropriate rates.

CCUs and umbilical cables

In addition to delivery of manipulated seawater to each under-ice chamber, a multi-purpose “umbilical” cable bundle also allowed various electrical connections between each chamber and the portable laboratory system located on the surface (as outlined in Fig. 2). Each umbilical cable had an overall length of 22 m, with the first 20 m protected by braided sheathing and the last 2 m unsheathed to allow for connection (i.e., of individual wires and tubes) to the various pieces of equipment. The umbilical cable was run through the ice-hole from the topside portable laboratory system.
down to its respective chamber, and was buoyed up under the ice, at intervals along its length, with small net floats (see Plate 1). Each incorporated two Teflon tubes (for built-in dual redundancy), two submersible electrical cables (one for a heating element, and one for an in-chamber circulation pump) and a cable for an in situ temperature probe. The CCUs at the end of each umbilical cable (containing element, pump, probe and supply tube outlets) were designed to push-fit tightly into a grommeted port (Uniseal®, 60 mm) in the side of each chamber (Fig. 3, but also see Fig. 1).

**Circulation and mixing control**

To avoid stratification of seawater within the chambers, we included an electronically controlled submersible circulation pump located in the CCU at the end of each umbilical cable. The amount of mixing was controlled by a combination of control voltage applied to the pump and the pump pulse rate which was set by a Zelio Smart Relay® (Model SR2B201BD) unit located in the portable laboratory control panel. We conducted tests prior to deployment to optimise the stirring velocities of the pumps, and determined that flow speed was proportionally related to the voltage applied (see Fig. 4). Stirring flows needed to be: (1) sufficient to homogenise the volume of water inside the chamber; (2) not so strong as to erode ice crystals or algae from the undersurface of the ice; and (3) comparable to ambient tidal current flows observed at the site. We used a flow velocity (~ 6.5 V, ~ 2 cm s⁻¹, Fig. 4) that was comparable to tidal current velocity at our study site (1–4 cm s⁻¹, unpublished data). The “pump and pulse” regime we used to avoid boundary layer formation (Miller-Way and Twilley, 1996) was three times 1 s full power pulses, every 5 min.

To monitor the circulation mixing pump function for each individual chamber, an analogue Ammeter was installed in the control panel in series with each pump’s power supply. If a pump malfunctioned, visual differences in the pump’s power draw characteristics were readily apparent. In addition, a remote wireless alarm was added to the system after a generator power failure in trial 1. The alarm indicated any issues relating to main power supply, water supply to the chambers, or any potential control panel malfunctions, allowing us to rectify problems in a timely fashion (i.e., before they cascaded through the system).

**Chamber water sampling and flux determination**

In flow-through incubation systems, fluxes of solutes are calculated from the differences in concentration between influent and effluent water. The concentration differences are a function of the water residence time in the chamber, which is controlled by seawater supply rate and chamber volume. Thus, fluxes can be determined using the following formula (Miller-Way and Twilley 1996):

\[
\text{Flux} = \frac{\text{Concentration}_{\text{outflow}} - \text{Concentration}_{\text{inflow}}}{\text{seawater supply rate}}
\]

In our case, we sampled the influent water from each individual Teflon tube delivering water to each replicate chamber. We did this by temporarily stopping the flow of water to each chamber, and then reversing the direction of peristaltic pumping to collect a 60 mL sample of the water.
present in each tube. To sample the “effluent” water, we allowed the peristaltic pump to run in reverse for a further 6 min (to flush all of the delivery tubing of influent water, and then to sample from the well-mixed chamber water) before retaining a 60 mL sample. With eight tubes connected to each of two peristaltic pumps, the sampling of influent and effluent water was quick (< 15 s for eight chambers simultaneously), nearly synoptic (all sampling completed in less than 15 min), and precise (i.e., was laboratory-based, allowing for immediate sample processing/preservation). This enabled calculation of fluxes in dissolved oxygen and inorganic nutrients (ammonium, nitrate, nitrite, reactive phosphorus, and reactive silicon) once a day.

In addition to measuring daily fluxes in dissolved oxygen and nutrient levels, derived from chamber influent and effluent seawater as described above, dissolved oxygen loggers (D-Opto®) were also mounted into grommeted ports (Uniseal®, 48 mm) in each chamber. Along with these each chamber also had an Odyssey® PAR and a Seabird® (SBE 56) temperature logger. Together these loggers gave higher resolution temporal data that allowed examination of the correspondence between daily periodicities of light, temperature, and photosynthetic oxygen production (Cummings et al. in preparation).

Assessment

The system was trialled at Cape Evans, Antarctica (77° 38.124’S, 166° 24.821’E) in November 2013. Manipulations of pH and temperature (orthogonally crossed treatments with replicate chambers arranged in a randomised block design) were maintained over a 6-day period (06–11 November 2013). The pH treatments chosen were ambient (pH ~ 8.00) and pH 7.75, and temperature treatments were ambient and ambient +0.4°C. Note that all pH values specified in this article are based on the total hydrogen ion concentration. A second trial, with five pH treatments (ambient, 7.80, 7.70, and 7.60) but no manipulation of temperature, was then conducted (12–17 November 2013). The following year, with slight improvements to our deployment methodologies and in an area with greater algal biomass, we successfully conducted a 14-day incubation experiment, with four pH treatments (ambient, 7.85, 7.75, and 7.60). This experiment conducted at Granite Harbour (77° 00.963’S, 162° 52.607’E) ran from 04 to 18 November 2014 (Cummings et al. in press). In all cases we deployed four replicate chambers for each treatment, again in a randomised block design.

Water samples were collected from the influent supplies to each chamber once daily, and the pH determined using a portable auto sampler coupled to a spectrophotometric analyser, using thymol blue dye as the indicator reagent (as described by McGraw et al. 2010).

Stability, accuracy, and precision of control systems

We examined stability, precision and accuracy of the two controlled parameters, pH and temperature, over the course of the two November 2013 trials and the main November 2014 experiment (Table 1). Although there was some variability in pH control probe readings (Fig. 5), due predominantly to the cyclical nature of the feedback control loop, there was also clear and statistically significant separation between pH treatments during testing (Table 1). Moreover, there was a strong linear correlation between the probe pH values in header tanks and the spectrophotometrically determined pH values in the inflow seawater being supplied to the chambers during the main experiment (R² = 0.97, p < 0.0001; Fig. 6). The relationship was not 1 : 1, and there was an average offset of 0.92 pH units over the pH range of 7.6 to 8.0 (Fig. 6). This offset was predominantly due to the use, in seawater, of standard laboratory pH probe calibration buffers (4.01, 7.00, and 10.01) required by the Omega controllers. Therefore, as previously described, we used seawater TRIS and AMP buffers to estimate a correction offset to set on the controllers to maintain our target pH values. Moreover we measured the actual pH values of seawater being delivered to each individual chamber, using an independent spectrophotometric method.

In situ control of seawater temperature (heating) was also examined for the eight heated chambers that were used in Trial 1. At the start of this trial, the eight temperature probes in heated chambers were all normalised to the ambient seawater (~1.92°C). This was done so that temperature changes could be measured, and therefore controlled, more precisely in situ with respect to ambient seawater temperature (Fig. 7; Table 1). Other than one pump failure which affected temperature in Chamber D for approximately 5 h, temperature was controlled and maintained at the target +0.4°C above ambient seawater in all eight heated chambers for the duration of this trial.

Chamber light effects

To determine if there was any significant effect of the transparent polycarbonate material the chambers were constructed from, on the light levels inside them relative to outside the chambers, we measured PAR using a hand-held scalar Biospherical® light probe. There was no statistically significant effect of the chambers on these light levels (pairwise Wilcoxon Rank Sum T-test, p = 0.403).

Discussion

This article has described a purpose-built system for investigating in situ responses of sea ice-associated ecosystems to future ocean acidification and warming, which has been successfully deployed and evaluated in Antarctica. This semi-enclosed system comprised specialised chambers, an above-ice portable control system for the precise control of seawater characteristics, and seawater delivery and sampling systems running between the chambers and the above-ice
Table 1. Estimates of accuracy and precision of controlled parameters in under ice chambers.

Chamber seawater supply pH control
Statistical comparisons made between treatment levels for the two trials and the main experiment. Values of chamber inflow seawater pH (daily means) read from control pH probe records, and actual daily inflow pH values determined from colorimetric (thymol blue) spectrophotometric analysis. The superscript Amb denotes ambient seawater (control) treatments, while the superscript numerical value denotes approximate target pH (e.g., /C24 7.7). Note all pH values are based on total hydrogen ion scale. For Trial 1, superscript H indicates that in situ seawater was heated by +0.4°C relative to ambient. Statistically significant differences between treatments are indicated by different letters (Tukey’s multiple comparisons test)

Trial 1. Cape Evans, 2013. See also Fig. 5A

| Header ID | Mean (n = 5) | SD | Tukeys (p < 0.05) |
|-----------|--------------|----|------------------|
| HT1 Amb   | 7.95 ± 0.00  | a  |                   |
| HT2 /C24 7.75 | 7.75 ± 0.01  | b  |                   |
| HT1 Amb   | 7.95 ± 0.00  | a  |                   |
| HT2 /C24 7.75 | 7.75 ± 0.01  | b  |                   |

Trial 2. Cape Evans, 2013. See also Fig. 5B

| Header ID | Mean (n = 4) | SD | Tukeys (p < 0.05) |
|-----------|--------------|----|------------------|
| HT1 Amb   | 7.95 ± 0.00  | a  |                   |
| HT2 /C24 7.80 | 7.79 ± 0.01  | b  |                   |
| HT3 /C24 7.70 | 7.62 ± 0.01  | c  |                   |
| HT4 /C24 7.60 | 7.45 ± 0.00  | d  |                   |

Main experiment. Granite Harbour, 2014. See also Fig. 5C

| Header ID | Mean (n = 14) | SD | Tukeys (p < 0.05) |
|-----------|--------------|----|------------------|
| HT3 Amb   | 7.90 ± 0.00  | a  |                   |
| HT4 /C24 7.85 | 7.77 ± 0.01  | b  |                   |
| HT2 /C24 7.75 | 7.67 ± 0.01  | c  |                   |
| HT1 /C24 7.60 | 7.50 ± 0.01  | d  |                   |

In situ chamber temperature control (°C)
Summary statistics for temperatures for the eight heated chambers; ambient seawater temperature measured adjacent to the chambers is also provided. Statistical comparisons (Holm-Sidak) of in situ temperatures (daily mean) for each of the eight heated chambers with ambient seawater are shown

Trial 1. Cape Evans, 2013. Note the asterisk* below for chamber D indicates this chamber was temporarily affected by a spike in temperature when the chamber control module was temporarily removed to replace a faulty pump. See also Fig. 7

| Chamber ID | Mean (n = 5) | SD | Maximum | Minimum | Difference | Holm-Sidak (p < 0.05) |
|------------|--------------|----|---------|---------|------------|----------------------|
| Ambient    | -1.903       | ±0.003 | -1.897  | -1.884  | -           | -                    |
| A /C24 7.75 | -1.460       | ±0.049 | -1.420  | -1.520  | +0.443     | Yes                  |
| H /C24 7.75 | -1.450       | ±0.046 | -1.420  | -1.520  | +0.453     | Yes                  |
| K /C24 7.75 | -1.437       | ±0.038 | -1.420  | -1.520  | +0.466     | Yes                  |
| N /C24 7.75 | -1.459       | ±0.052 | -1.320  | -1.520  | +0.444     | Yes                  |
| D* /C24 7.75 | -1.451       | ±0.157 | -1.320  | -1.920  | +0.452     | Yes                  |
| G /C24 7.75 | -1.451       | ±0.047 | -1.420  | -1.520  | +0.452     | Yes                  |
| J /C24 7.75 | -1.432       | ±0.036 | -1.320  | -1.520  | +0.471     | Yes                  |
| M /C24 7.75 | -1.433       | ±0.068 | -1.220  | -1.520  | +0.470     | Yes                  |
control system. The system worked well in the cold water, under sea ice environment; the chambers remained firmly attached to the under surface of the ice and were well sealed from the underlying waters, while allowing delivery of external seawater to the chambers at required flow rates and pH levels (Fig. 5, Table 1). Heating of the water within the chambers could also be effectively controlled (Fig. 7, Table 1). Moreover, sampling of the chamber waters from above the ice was efficient and did not disrupt the conditions within the chambers.

All main system components proved reliable during deployment (specifications of main systems components are given in Appendix). Both temperature and pH controllers, and their respective probes, were stable in the cold conditions of Antarctica, and there were no failures of any of these devices. Failures of in situ equipment (a total of 6 circulating pumps in the CCUs failed over the 2 years of deployments) were quickly detected and corrected using an alarm and removable CCUs, respectively. Importantly, the “plug and play” design of the CCUs enabled divers to quickly replace faulty components in-situ. Also importantly, even when circulating pumps failed, the configuration of our system was such that inflowing water and therefore pH treatments were maintained, albeit at lower rate of mixing.

Fig. 5. Manual control (probe) pH recordings (from each pH controller for (A) Trial 1 (6th to 10th November 2013) with two pH levels supplied from header tanks HT1 and HT2, (B) Trial 2 (13th to 16th November 2013) with four pH levels supplied from header tanks HT1–HT4, and for (C) main experiment (3rd to 18th November 2014) supplied from header tanks HT1–HT4. All pH values are based on total hydrogen ion scale. Note that the vertical dashed lines in the three plots represent the period of time for which daily mean pH values were calculated in Table 1.

Fig. 6. Manual pH controller probe recordings (daily mean values) from chamber inflow supply header tanks (HT1–HT4) vs. daily spectrophotometric pH values for influent seawater sampled from each chamber sampled during the main experiment (4th to 17th November 2014). Note all pH values are based on total hydrogen ion scale. The 95% confidence intervals are shown in blue and the slope of 1:1 is shown as the dashed line.

Fig. 7. Mean temperature values for four replicate chambers at the two pH treatment levels, ambient pH chambers (A, H, K, N) shown with blue symbols and reduced pH chamber (D, G, J, M) shown with red symbols. Ambient temperature (−1.92°C) shown as a black trace. The vertical dashed lines indicate when chamber heaters were turned on and off. The * indicates a spike in temperature when chamber control unit (CCU) in chamber D was removed from the water by divers to replace a faulty pump.
Alternative options for pumps exist (e.g., high specification Rule or Sea-Bird pumps). Similarly, while the temperature and pH controllers used worked well, other brands (e.g., dTrans 02, with internal data logging capability) could allow the customised use of more appropriate seawater pH buffers (e.g., TRIS and AMP) and expand system capacity, for example, enabling automatic simulations of natural diel periodicity in the parameter of interest.

With this custom-built system, both seawater pH and temperature were precisely controlled and maintained, in situ, in a challenging field setting in Antarctica, enabling us to assess the productivity and functioning of an intact under-ice microalgal community that is practically impossible to recreate in a conventional laboratory setting. With minor improvements made after the two trials in 2013, we successfully conducted a 14-day experiment in 2014 in which we maintained four distinct and sustained pH levels. On a final practical note the incubation chambers were designed to “nest” within each other to save transport space, where fitting the whole system (control panel, header tanks and incubation chambers), into the smallest space and with the least weight possible, was a vital consideration given the remoteness of our two sites.

Looking to the future, the flexible design allows for the possibility of multiple experimental designs involving combinations of pH and temperature. The system could also easily accommodate other seawater treatments, so long as they can be maintained reliably in header tanks and distributed in suspended or dissolved forms to the chambers (e.g., phytoplankton, suspended sediments, organic matter, inorganic nutrients, toxicants, etc.). In addition to the application described above, the chambers could also be used in benthic habitats by “inverting” them, although obviously depth would be limited to the length of umbilical cables used. We suggest they could also be used in temperate, sheltered systems with the portable field laboratory established on a dock, barge, or ashore. This flexible system has many possibilities for ocean acidification research, or indeed questions relating to other environmental stressors faced by coastal communities in challenging environments.

**APPENDIX**

| System component              | Manufacturer | Model       | Main specifications                                                                                                                                 |
|-------------------------------|--------------|------------|----------------------------------------------------------------------------------------------------------------------------------------------------|
| pH control probes            | Sensorex     | Sensorex S150C | Polycarbonate body  
0–14 pH Na⁺ error > 12.3 pH  
Response Speed pH > 90% in 1 second  
Pressure rating 50 psi  
Wetted materials: polycarbonate, silicone, platinum |
| Temperature control probes   | Servotech    | QR16G, PT-100  | 4-wire  
R0-drift 0.04% after 1000h  
0.4 K/mW at 0°C |
| pH controllers               | Omega        | PHCN-37-AI-230 | Resolution: 0.1, 1 mV  
Accuracy: ± 0.1 mV @ 25°C  
pH range: 0–14.00 pH (620 mV to −620 mV)  
Resolution: 0.01 pH, 0.1°C  
Accuracy: ± 0.01 pH; ± 0.5  
Input impedance: > 10¹²Ω  
Automatic temperature compensation  
Operating temperature: 0–50°C |
| Temperature controllers      | Omega        | CN742      | Accuracy: ± 0.25% span, ± 1 least significant digit  
Operating temperature: 0–50°C |
| Intake and circulating pumps | Whale        | GP8825     | Submersible inline pump  
Body diameter: 36 mm  
Body length: 97 mm  
Operating voltage: 24Vdc  
Current draw: 1.4-3.1A  
Maximum head: 10 m  
Maximum flow rate: 3.5 gallons per min  
Semi-continuous duty |

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Appendix (Continued)

| System component     | Manufacturer                  | Model             | Main specifications                                      |
|----------------------|--------------------------------|-------------------|----------------------------------------------------------|
| Pinch valves         | BioChem Fluidics               | 100P3MP24-025     | Operating voltage: 24Vdc                                  |
|                      |                                |                   | Power: 4 W                                                |
|                      |                                |                   | Two-way operation                                         |
|                      |                                |                   | Tubing size: 1.6 mm                                       |
|                      |                                |                   | Operating pressure: 20 psi                                |
|                      |                                |                   | 300 W, 24 V                                              |
| Heating elements     | Argus Heating Ltd              | Cartridge heater  | Pocket type: Stainless steel 316                         |
|                      |                                |                   | Diameter: 16 mm                                           |
|                      |                                |                   | Length: 200 mm                                            |
|                      |                                |                   | 50 mm cold zone                                          |
| Peristaltic pumps    | Masterflex Ltd                 | 7528-10           | L/S modular digital drive                                 |
|                      |                                |                   | Speed 6–600 rpm                                           |
| Peristaltic pump heads | Masterflex Ltd             | 7536-04           | L/S pump heads                                            |
|                      |                                |                   | 4 channel to suit 24 tubes                                |

Control Panel specifications.
The main control was constructed in IP65 rated Gewiss GW46-QP (800 mm × 585 mm × 300 mm) fibreglass panel with a clear lid. For any control wiring detail please contact the corresponding author.

Other system specifications

Built components

Under-ice chambers
Unit volume: 144 litres
Number of units: 17 (one spare)
Main construction materials: 2 and 5 mm polycarbonate
Unit bare weight 3.5 kg

Header tanks (times 4)
Available off the shelf and cost effective. Inherent insulation properties and chemically inert ABS plastic

Pinnacle 55L Eskimo ice chest
Fitted with 15 mm Apex Space Saver ballcock valve

Silicon tubing for diffusion coils
Chemically inert with good gas diffusivity. Note that while gas non-gas permeable silicon tubing is available it not desirable for this application

Chamber circulating (flow) regime
Completely customisable using some form of PLC or smart relay such as the Zelio product

Pulse duty and duration: infinitely variable by PLC
Continuous setting: variable in range ¬1–8 cm s⁻¹

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Conflict of Interest

None declared.