

## A comparison of two pH-stat carbon dioxide dosing systems for ocean acidification experiments

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### Abstract

As the oceans acidify due to increasing atmospheric CO<sub>2</sub>, there is a growing need to understand the impact of this process on marine organisms. Field observations are difficult because of multiple covarying factors (e.g., temperature, salinity). As such, there is interest in conducting controlled, laboratory experiments to best understand how changes in acidity will affect marine organisms. We tested two intermittent CO<sub>2</sub> dosing systems, a “home aquarium hobby” grade pH controller and an industrial process control platform. We assessed stability, accuracy, and precision over 7-d experimental periods as well as relative cost of the two configurations. We also compared three laboratory-grade pH electrodes to the hobbyist electrode to further evaluate electrode quality on system-controlled pH stability and drift. Whereas the industrial system offered some benefit with regard to autonomy, our results show that the low-cost hobbyist system can be modified appropriately to provide comparable pH control. We provide a detailed list of procedures and software developed for the implementation of a cost-effective, precision-controlled CO<sub>2</sub> dosing system to support laboratory-based ocean acidification experiments.

The pH of the ocean is declining in response to increased concentrations of atmospheric carbon dioxide, [CO<sub>2</sub>]. This process, known as ocean acidification, consequently changes carbonate system equilibria resulting in greatly declining carbonate ion availability. Calcium carbonate mineralization is thermodynamically limited by carbonate ion activity ( $\alpha\text{CO}_3^{2-}$ ). Therefore, ocean acidification is expected to severely limit growth of calcified structures. Organismal response (by calcifiers and non-calcifiers) to ocean acidification is the subject of many recent studies (Widdicombe and Needham 2007;

Checkley et al. 2009; Ries et al. 2009; Wilson et al. 2009; Cummings et al. 2011; Kroeker et al. 2011; Munday et al. 2011; Doropoulos et al. 2012; Ferrari et al. 2012; and references therein). The results of these studies demonstrate that the impact of ocean acidification on marine life is not restricted to calcified structures. Other physiological responses range from changes in growth rate and morphology to changes in gene expression. Whereas there remains a vast knowledge gap with respect to impacts of ocean acidification on marine biota, entry into this area of research may be hampered due to prohibitive cost and lengthy method development required to precisely control aquaria pH in the laboratory.

It is possible to control seawater pH in the laboratory through a variety of means. For example, synthetic seawater can be produced and tailored to desired dissolved inorganic carbon (DIC), total alkalinity (A<sub>T</sub>), and pH levels. These parameters can be maintained by minimizing gas exchange and evaporation (Gattuso et al. 1998). The pH of natural waters can be adjusted with mineral acids and bases to simulate historic and modeled pH (Riebesell et al. 2000; Spero et al. 1997). This approach, however, is generally avoided because of its impact on alkalinity and total organic carbon (Dickson et al. 2007). More recently, adjustments of pH using continuous dosing of natural waters with

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premixed specialty CO<sub>2</sub> enriched air mixtures (CO<sub>2</sub> + air) enable a broad array of laboratory experiments. Indeed, this approach is used successfully in conjunction with moderate- to large-scale recirculating flow-through seawater systems (Checkley et al. 2009; Ries et al. 2009) as well as static exposures (Iglesias-Rodriguez et al. 2008). This approach is simple and should, in theory, reduce variability in set pH values over an extended period. Whereas this method allows for little deviation in tank pH, specialty mixed gases can be costly. Mass flow controllers may be used to mix incoming air to a desired pCO<sub>2</sub> (de Putron et al. 2011; Edmunds et al. 2012). Whereas this method assures a constant pCO<sub>2</sub> value entering the aquaria, equilibrium pH within the aquaria may shift depending on biological activity and/or passive gas exchange. An alternative gas dosing approach uses intermittent dosing with pure CO<sub>2</sub> gas along with continuous aeration of tanks with ambient air. This approach can be used to control pH in individual aquaria, as static or static renewal exposures (Gazeau et al. 2007; Waldbusser et al. 2011), or with header tanks that provide flow-through to one or more individual aquaria (Berge et al. 2006; Munday et al. 2009; Munday et al. 2011).

We assessed two approaches to intermittent CO<sub>2</sub> dosing of static aquaria. Specifically, we evaluated two of the many available dosing systems with respect to stability, accuracy, precision, dependability, and cost, as well as pH electrode performance, and describe their deployment in detail. We chose to compare an industrial system manufactured by Omega Engineering and a hobbyist system manufactured by Digital Aquatics. Both systems monitor the pH of aquaria using a pH probe and have a controller for which a specific target pH value can be set. In both systems, continuous aeration of the aquaria using ambient air leads to outgassing of CO<sub>2</sub>, causing an increase in pH. Once the upper pH threshold is met, the controller opens a gas solenoid valve, allowing CO<sub>2</sub> gas from a pressurized cylinder to be bubbled into the aquarium water, thereby lowering pH.

## Materials and procedures

### Omega Engineering (OE) system configuration

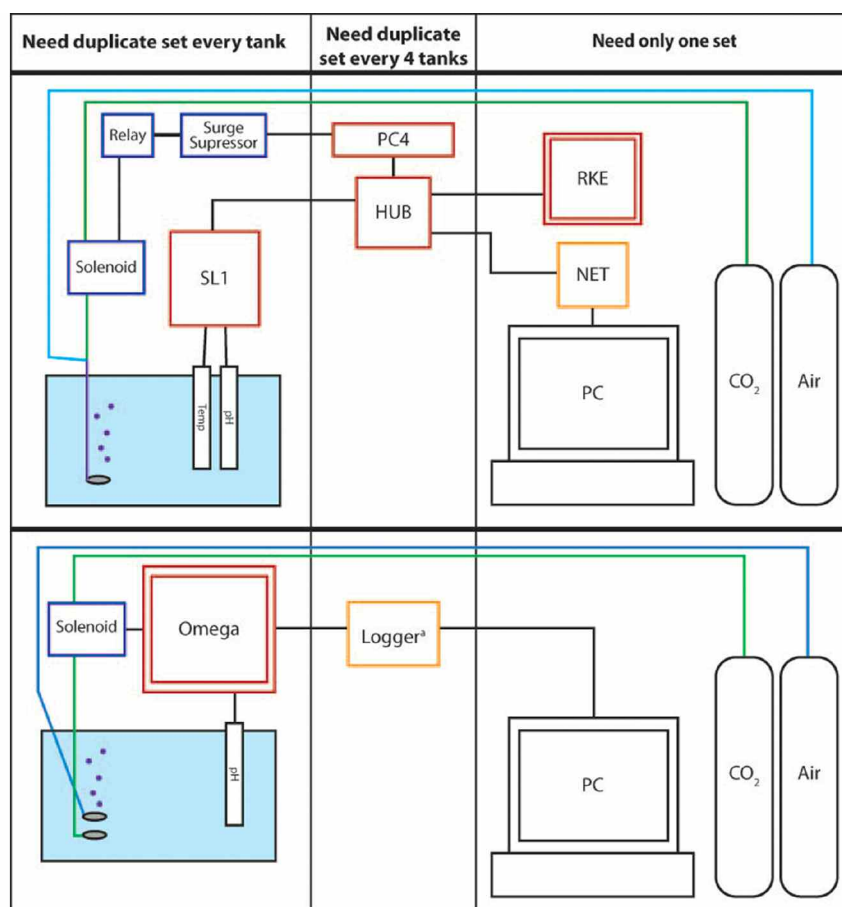
The Omega Engineering single setpoint proportional pH controller (PHCN-901) is an industrial process management platform that has a splash and corrosive resistant enclosure. This unit has a standard connection for pH electrodes and allows for a broad performance range of electrodes. Additionally, the unit sends data to an external logger over analog amperage output. The controller houses all functions required for control of one aquarium, and therefore is replicated for each additional tank. pH calibration is done prior to sealing the housing, using a two-point calibration method. Calibration dials (analog potentiometers) consist of an “offset” and “slope” set. Any set of pH calibration standards may be used, allowing the system to use either National Bureau of Standards (NBS) standard pH scale buffers (pH<sub>NBS</sub>) or synthetic seawater pH buffer solutions (pH<sub>s</sub>) (Byrne 1987; Millero et al. 1993a, 1993b).

In experimental aquaria (Fig. 1), pH is maintained at the given setpoint by CO<sub>2</sub> dosing when the pH of the aquaria drifts above the setpoint value by approximately 0.01–0.02 pH units. A dosing schedule circuit within the controller allows optimization of system hysteresis and offers the ability to dose intermittently via a gas solenoid valve (Omega SV3204) that is plumbed to a CO<sub>2</sub> cylinder. Multiple controllers can feed into the same data logger. We used an Omega four channel 25 mA current data logger (omega.com; OM-CP-QUADPROCESS-25MA). Data collection and storage times depend on the choice of polling rate. In our study, we used a 5-min polling rate allowing for collection of 4 channels of data for more than 2 weeks. We downloaded data every 3–4 d corresponding to the schedule of water changes for the aquaria. Multiple gas solenoid valves can be plumbed to the CO<sub>2</sub> cylinder using standard gas connections. A step down regulator (swagelok.com, KPR1DFC412A20000), attached as a “third stage” to a standard two-stage laboratory CO<sub>2</sub> regulator, was set to 2–7 psi to lower flow rates delivered to the aquaria.

The output line from the gas solenoid was connected via an in-line check valve to a standard aquarium airstone placed directly below the continuously flowing ambient air airstone at the bottom of the aquarium. This tiered airstone setup promotes water circulation in the aquarium and assists in efficient gas diffusion. We used commercially available software (omega.com, OM-CP-IFC200) specific for the data logger to manually control start and stop of data logging. We found that connecting a laptop computer via a USB port to the four-channel Omega data logger resulted in a 0.90 mA voltage drop in the recorded signal. Hence, we connected the computer to the system for short periods of time and stopped data logging until the data could be downloaded. The computer was not connected to the data logger during electrode calibration or long-term monitoring of aquaria pH. During electrode calibration, we found that the data polling rate had to be changed to allow sufficient time for the data logger to record mA output during each calibration step to externally verify pH calibration. The data polling rate was set to 2-s intervals during pH calibration. Afterward, we reset the polling rate to 5-min intervals for long-term pH monitoring. We regressed calibration data logs offline to convert mA output to pH. We optimized hysteresis compensation using the two analog potentiometers (“Proportional Settings”). Adjusting these settings changes the total on/off time per dose as well as frequency of the cycle. Manual adjustment of CO<sub>2</sub> dose can be done, as well, by changing the gas pressure to the solenoids using the ‘third stage’ gas regulator as mentioned above.

### Digital Aquatics (DA) system configuration

The Digital Aquatics (DA) ReefKeeper Elite controller (RKE) is an aquarium hobbyist system (Table 1). The RKE platform allows control/monitoring of several water quality variables depending on the choice of an appropriate module from a host of add-on modules. Indeed, this controller is most often used in a hobbyist reef tank system and is capable of automating



**Fig. 1.** Top: Digital Aquatics, Inc. (DA) ReefKeeper Elite (RKE) setup, showing required duplication. Bottom: Omega Engineering (OE) setup, showing required duplication. Note that the OE system log is generated at each head unit box, but temperature data is not logged as is possible with the DA system. OE logger (a), as shown, is a 4-port unit, and therefore a system must be deployed for every four tanks. Components are color coded as follows: red – controller, blue – gas dosing, orange – logger.

**Table 1.** Qualitative assessments of comparative strengths and weaknesses. Stability scores reflect system time between pH electrode calibrations. User customizable scores are determined by optional modules available for end user. Memory capacity scores reflect time between logger downloads. Memory capacity of the DA system is rated highly when using the RSS feed fetching script because it is only limited by computer hard disk space. User-friendly scores are reflective of ease of deployment with provided product manuals. Cost scores are reflective of cost/aquarium deployment at larger scales. Overall performance scores represent the ability of the system to perform ocean acidification experimentation adequately.

	OE	DA
Out-of-the-box	+	-
Stability	+	+
User customizable	-	+
Memory capacity	+	+
User friendly	-	-
Cost	-	+
Overall performance	+	+

most reef tank functions (pH, temperature, oxidation reduction potential, and salinity). Specifically, lab modules (SL1 or SL2) monitor pH over a BNC connection, allowing flexibility in pH electrode selection. In addition to pH electrodes, temperature, ORP, and salinity probes are available and can be attached to the lab units. The DA system is highly modular, as one controller unit (RKE) can address 255 peripheral units.

Similar to the OE controller, calibration of pH uses a two-point calibration where the user is able to digitally set each calibration target. This allows for the use of synthetic seawater buffers (Dickson et al. 2007) for enhanced pH accuracy. pH is controlled by programming a “function” for each power outlet of a 4-port power module (PC4) by comparison to the measured pH of the SL1 or SL2 lab module. Assigning a simple “function” allows the user to select power on/off criteria for the 120V AC relay wired to a 120V AC gas solenoid valve. pH is controlled through direct injection of CO<sub>2</sub> from a compressed gas tank into the experimental aquaria.

In our configuration, each system lab unit included an external flasher relay (i.e., grainger.com; cat# 5JJ48) fed into a

120V, normally closed, gas solenoid valve (alliedelect.com; cat# VX2330-02T-3CR1). This allowed for the opening/closing of the gas solenoid. The flasher relay was set to a rate of 0.25 s on, 3 s off. The voltage drop associated with turning on the solenoid valve produced a power surge that resulted, at times, in a software restart and accompanying loss of pH control. To remedy this problem, single outlet surge suppressors (amazon.com; cat# B00006B81E, B000EWVSZK) were wired between the external flasher relay and the outlet in the PC4 strip serving each aquarium. CO<sub>2</sub> gas routing was identical to the OE system. A web interface unit (NET) delivered data via an ethernet connection to attached computer. Data were logged externally over RSS feeds provided by the NET interface at 1-s intervals. Data were backed up to external and cloud storage for off-site verification at 1-h intervals. The Perl and batch scripts we used are available in the supplemental materials.

## Assessment

### System stability tests

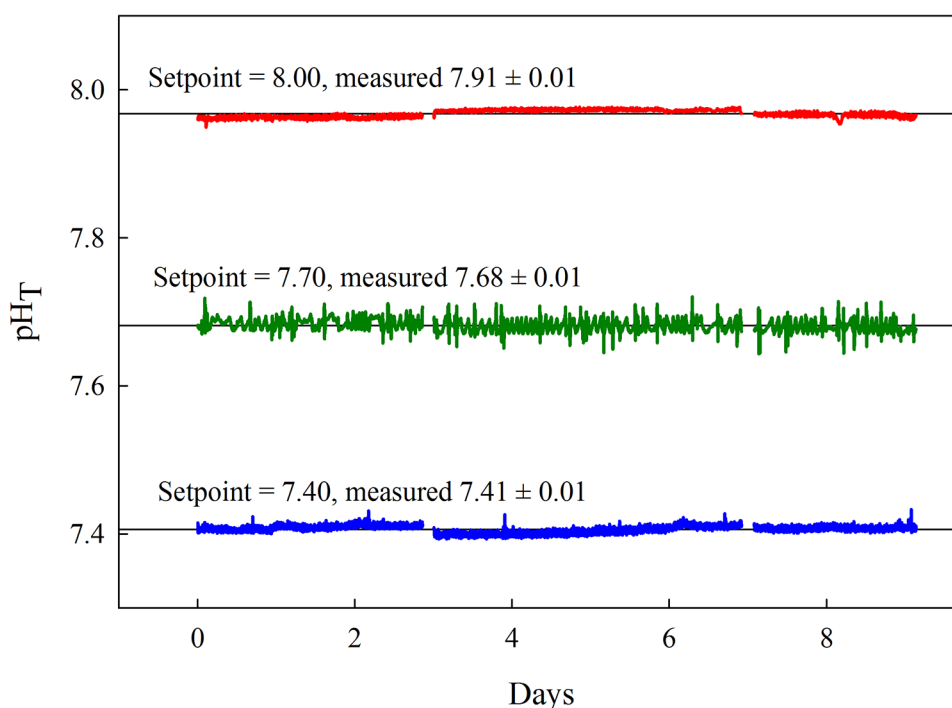
We completed a 7-d pH stability test on each system using comparable pH electrodes (OE: Ross Combination pH electrode, Thermo Scientific, cat# 8256BN; DA: Orion PerpHecT combination pH electrode, Thermo Scientific, cat# 13-642-559). During this test, we set the pH controller systems at specific pH levels and held the pH for the duration of the experiment. For the OE system stability experiment, we used filtered (1.0 μm) and UV sterilized seawater from Savin Hill Cove, Boston, MA. We set the OE controllers to a 1 control and 3 experimental pH values (pH<sub>T</sub> = 8.0, 7.7, and 7.4). We down-

loaded data logs weekly. Due to the cost of OE controllers, we did not replicate aquaria (Fig. 2). For the DA experiments, we used filtered (1.0 μm) and UV sterilized seawater collected from Mt. Hope Bay, RI. Due to the lower cost of the DA units, we replicated the aquaria ( $n = 4$ ). DA systems were set to control pH as in the OE experiment. RSS feed provided for continuous data downloading (Fig. 3).

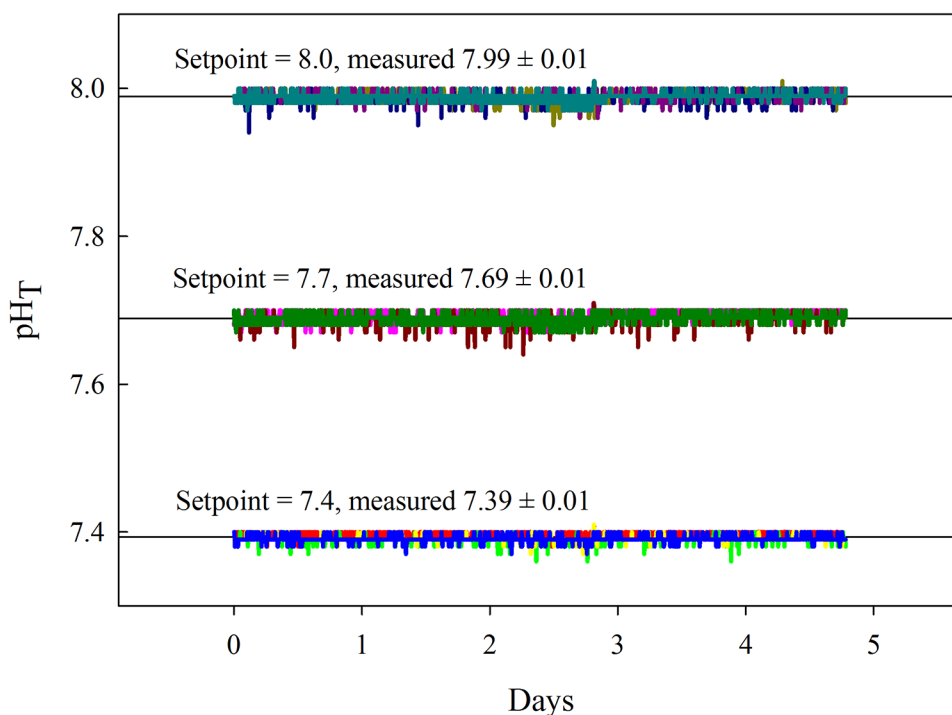
For stability comparisons, we compared pH data collected every 5 min across 5 days by both systems. Using the value of observed pH – expected pH, we compared the data such that positive values indicate the system is reading higher than expected and negative values correspond to lower than expected. To assess the variability in reading, we calculated the standard deviation for each replicate. The DA replicates were tested for statistical similarity with a one-way ANOVA, and the standard deviation value for each OE replicate was compared to the 95% CI for the corresponding DA pH treatment group. There was no significant difference in the standard deviation between DA replicate aquaria at the different pH treatments ( $F_{2,9} = 3.13, P > 0.05$ ). The standard deviations of the OE treatments were within the 95% CI for the DA treatments (Fig. 4).

### pH electrode stability tests

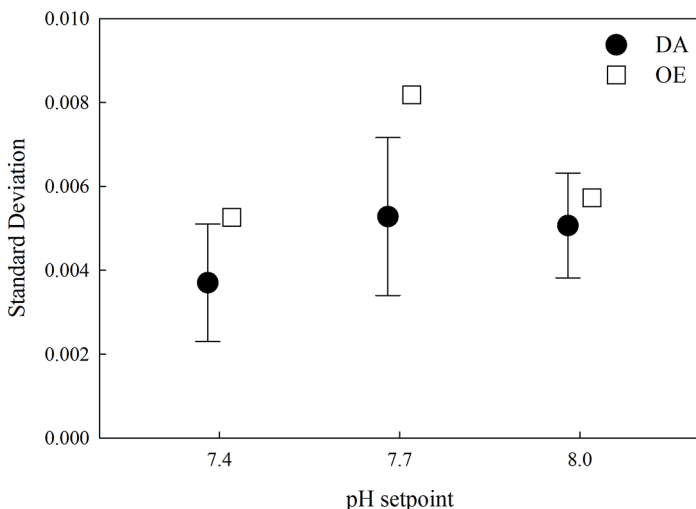
Because our goal was to identify cost-effective alternatives to support ocean acidification experiments, we assessed the stability of a range of pH electrodes with the DA controller. For electrode testing, we used filtered (1.0 mm) and UV-treated seawater from Mt. Hope Bay, RI, USA. We buffered total alkalinity (A<sub>T</sub>) to 2500 μmol/kg using sodium carbonate to minimize pH change over the study period. We verified A<sub>T</sub> follow-



**Fig. 2.** OE pH controller stability experiment time series using Thermo Scientific Ross combination pH electrodes. pH<sub>T</sub> setpoints (mean ±SD) are shown.



**Fig. 3.** DA pH controller stability experiment time series using laboratory Ag/Ag-Cl refillable electrodes. pH<sub>T</sub> setpoints (mean ± SD) are shown. Each setpoint consists of four experimental aquariums represented by contrasting colors.



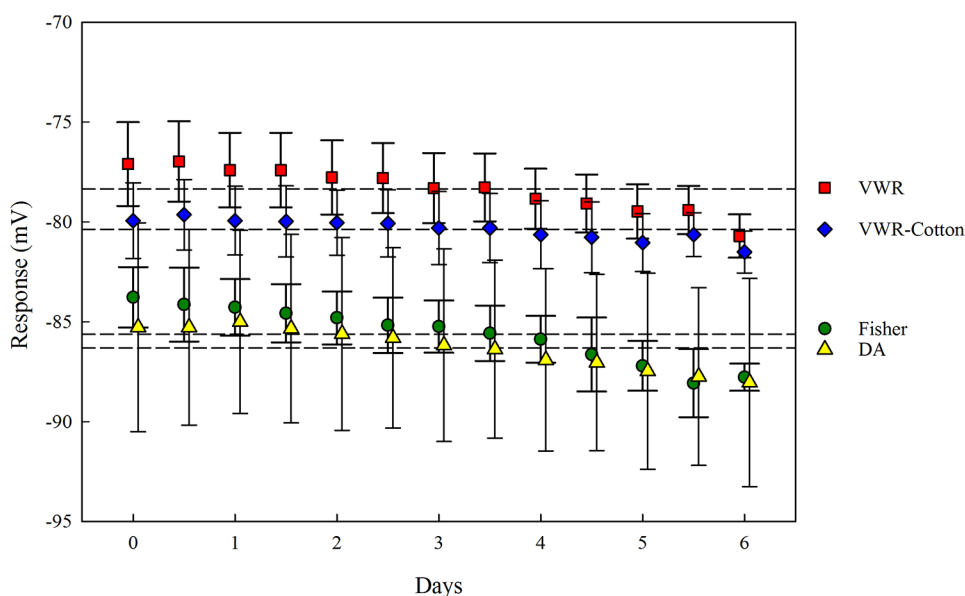
**Fig. 4.** Performance comparison between DA and OE systems for 5-d stability experiments. Error bars represent DA 95% CI ( $n = 4$ ). OE standard deviation means do not fall within the DA 95% CI for treatment groups pH = 7.4 and 7.7 whereas the SD mean for OE pH=8.0 treatment group falls within the corresponding DA 95% CI. Precision of control appears to be comparable, therefore for pH = 8.0 treatment group only.

ing standard methods (Dickson 1981). We added the buffered seawater to 40 L glass aquaria and aerated the aquaria with house compressed air. Twice daily, we verified controller pH using an independent hand-held pH meter (Hach H170G). We calibrated both the DA system and the Hach meter on the

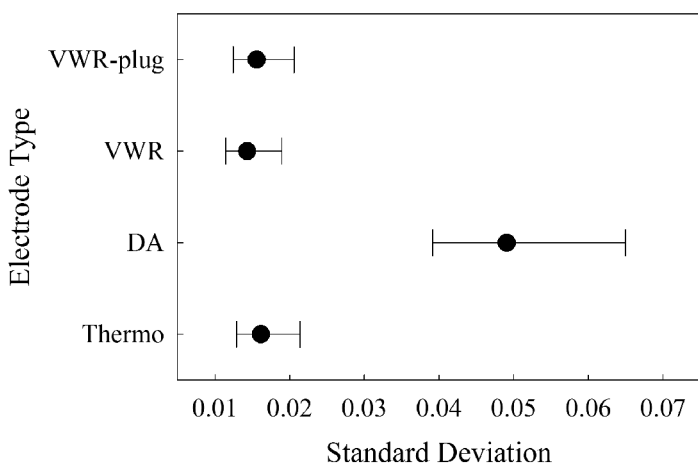
total hydrogen concentration scale (pH<sub>T</sub>) using colorimetrically calibrated synthetic seawater buffers (Byrne, 1987). In the case of the Hach handheld meter, which collects mV readings, we calculated pH offline due to software restrictions. We measured pH<sub>T</sub> directly twice a day over each 7-d experiment.

We tested pH electrodes available from Digital Aquatics (DA), Inc. (sealed, non-refillable epoxy-body electrodes). We also compared calibration drift of the DA electrodes to laboratory-grade Ag/Ag-Cl combination pH electrodes (Fisher Scientific, Cat#: 13-642-559; VWR International, Cat#: 14002-780). Since the refillable electrodes from VWR exhibited rapid reference solution evaporation, we tested an additional set of VWR pH electrodes fitted with a cotton pad covering the electrode vent (VWR-plug). We tested each electrode (DA, Fisher, VWR, VWR-plug) on two separate metering devices (DA digital readout and Hach H107G handheld meter). This allowed for comparison between replicate tanks with the same meter, between different meters, and between electrode manufacturer (e.g., VWR, VWR-plug). DA electrodes had a larger mV reading range (10.4 mV) than did the laboratory grade electrodes (Fisher = 6.6 mV, VWR = 6.3 mV, VWR-plug = 3.7 mV) (Fig. 5). Because repeatability in replicate electrode response indicated comparable precision, a test for Equal Variances showed not all electrodes exhibited comparable variance (Fig. 6) (Levene's Test,  $P < 0.001$ ,  $W = 52.40$ ,  $\alpha = 0.05$ ,  $n = 12$ ,  $k = 4$ ).

Once calibrated, the relationship between pH readout and electrode potential should remain constant. However, normalizing the SL1 pH readings to mV readout indicated a



**Fig. 5.** Raw mV electrode potential output from pH electrodes as measured by Hach H107G with standard deviation across individual electrodes of same type. The mean of three aquaria setups per electrode type (mV) is represented by a horizontal line. Three replicate tanks for each probe as measured are represented with respect to time (mean ± SD): Fisher (−85.6 ± 1.8); DA (−86.3 ± 4.1); VWR (−78.3 ± 1.8); VWR-plug (−80.4 ± 1.5)



**Fig. 6.** Electrode response comparisons (standard deviation of electrode response, N = 12) with 95% confidence interval bars. DA electrodes exhibited significantly higher variance in electrode response (Levene’s Test,  $W = 52.40$ ,  $P < 0.001$ ,  $\alpha = 0.05$ ,  $N = 12$ ,  $k = 4$ ).

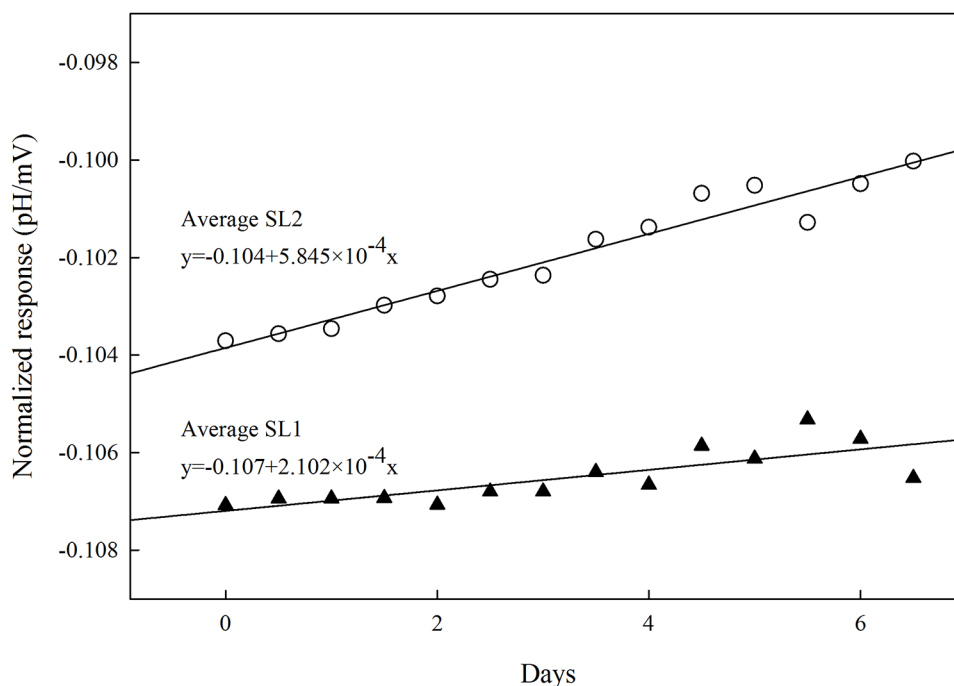
pH/mV ratio shift independent of calibration procedures on all units (Fig. 7). When comparing SL2 unit performance under identical data collection conditions, calibration drift was more pronounced than that of SL1 units. Because we accounted for electrode drift in the data normalization procedure, we suggest that differences in slope were likely caused by the electronics used in the lab unit. Linear regression of normalized values indicated that the calibration of the SL2 drifts at a significantly faster rate than that of the SL1 unit (ANOVA,  $F_{1,24} = 36.34$ ,  $P < 0.001$ ) (Fig. 7).

### Cost analysis

Digital Aquatics, Inc. (DA) ReefKeeper Elite-based systems offered a cost-effective alternative to industrial controllers such as those offered by Omega Engineering (OE). The cost for a single tank at the time of this study was \$1057 for the DA system and \$1180 for the Omega system. However, replication favored the DA system as each additional tank was \$408 for the DA system but \$906 for the Omega system.

### Discussion

Over the period of the experimental trial, we scored each system for a handful of qualitative parameters (Table 1). The low gas flow rate required for ocean acidification experiments to rear sensitive marine larvae makes control of aquaria pH very difficult. The dosing and sensor equipment can be costly, and thus we compared two systems. Each system offered unique advantages and disadvantages. While precision of the pH control by the “out of the box” DA hobbyist system was less than that of the OE industrial system, we found that the addition of external components to the DA system, such as flasher relays and individual surge protectors, yielded precision control on par with the more costly OE system. This, along with the cost savings, suggests the DA system is a viable option for ocean acidification studies. The biggest strength of the OE system over the DA system was its variable dosing schedule. The OE system was able to pulse-dose the aquaria with variable on and off times through the proportional control knob-driven potentiometers. Without external control, the DA systems dosed continuously until the aquarium pH dipped below the target pH set point, resulting in excessive



**Fig. 7.** Normalized response (pH/mV) highlighting DA SL2 ( $R^2 = 0.947$ ) vs. SL1 ( $R^2 = 0.627$ ) electrode calibration drift. Note that the slope of each line indicates SL2 units exhibit calibration drift 2.8× the rate of the SL1 units using same pH electrode (Fisher).

system pH lag and subsequent hysteresis. By adding variable timer relays between the power outlet and solenoid valve to the DA system, OE pulse dosing could be mimicked by the DA system.

Power interruption impacted the DA system. Preliminary DA data logs contained gaps ranging from 30 min to several hours, which were likely a result of power surges and/or power loss. Locally stored data logs appeared to be corrupted during such events. Upon power loss, SL2 units took up to 15 min to read correct pH, whereas the SL1 units recovered nearly immediately. We ruled out slow electrode response as the issue since we tested electrodes using an external handheld meter (Hach H107G). These tests showed equilibration of signal readings within 5-10 s. To remedy data corruption and electrode stability problems, we placed the systems on uninterruptable power supplies with battery backup and line conditioning and connected to power lines with emergency generator power. We enabled the NET unit RSS feed feature collected data at 1-s intervals using a Perl script. Using this approach, we recorded 3 months of uninterrupted data, including data logs recorded during brownouts, blackouts, and line surges associated with Hurricane Sandy.

Calibration of electrodes on the DA system revealed some issues with the SL2 unit. Calibration of all electrode on SL2 units was slow and cumbersome due to the slow response of the electronics (20 min/tank). We could, conversely, complete calibration on the SL1 units in a time frame comparable to the OE system (< 4-5 min per controller). We evaluated electrode equilibration using a Hach pH meter and found equilibration time

to be within expected limits (5-10 s). This indicated that response time was due to the SL2 electronics not the pH electrodes themselves. Stability results show that while the SL2 systems maintained setpoint pH, calibration needs to be monitored and updated daily (Fig. 7). When using SL1 units, we increased the calibration cycle to 3 days of operational accuracy (a set point of 0.02 pH units). In contrast, the OE system did not require pH calibration adjustments over 7-10 d periods.

Importantly, DA system calibration must be run manually on each tank, one tank at a time. This is in contrast to the OE system with its dedicated controllers for each tank. The OE setup allowed for simultaneous calibration of multiple tanks, and therefore, significantly reduced system downtime during calibration.

### Comments and recommendations

Systems provided an exceptionally stable platform for relatively automated experiments with minimal modification. With the modifications presented here, we found the DA system provided comparable precision and accuracy of control. Additionally, the modular nature of the DA system allows for a great amount of user customizability whereas the OE system allows for a set number and type of data to be collected.

In designing systems, power backup and data logging must be addressed, and the approach will depend on the specifics of the laboratory location. Whereas OE controllers offer more independent control with high “out-of-the-box” stability, the relative expense of such industrial controllers may prohibit purchase. Researchers will have to balance the relative costs and

benefits of the OE and DA systems to determine the best fit for their research needs. The DA system, through modification and regular monitoring of performance, offers a cost-effective alternative to expensive pH controller infrastructure, and can, as shown here, produce results comparable to the OE system.

The cost to set up the first tank, using either system, is nearly identical. However, OE systems become more expensive, averaging almost double the cost per tank when replicating over eight systems. Because true replication to assess pH/pCO<sub>2</sub> effects requires individually controlled tanks, cost of the experimental system heavily favors the DA controller. Although the statistical results show significant differences in tank pH control, the observed differences are outside the precision range of the pH metering system. DA SL1 performance, including calibration drift, is better than that of the SL2 units. Based on our results, we recommend using the SL1 units when employing the DA system.

Appropriate selection of pH electrode is important because it determines pH measurement precision. We found no statistically significant difference in accuracy on the OE or DA systems using laboratory grade Ag/Ag-Cl combination pH electrodes. However, the DA hobbyist electrode was not as precise as the laboratory grade electrodes. Therefore, we suggest that laboratory grade electrodes be used, although it is unnecessary to use electrodes with higher precision than  $\pm 0.01$  pH units.

In DA system experiments, we encountered calibration drift that required recalibration every 3 days. For this reason, if using a DA system, we suggest that pH be confirmed with an externally calibrated meter daily to monitor for drift. Since refillable electrodes suffer from solution evaporation over time, the observed drift in these electrodes on the DA system could be exacerbated by particularly warm or dry conditions and so users should develop a drift monitoring protocol for their laboratory conditions. For our purposes, a pH calibration divergence of 0.02 units prompted a recalibration procedure. We found it necessary to recalibrate the electrodes every 5 days on average. In our study, we also added precautionary protocols to replace all pH buffers after being used for system calibration due to the heavy use required (20 aquaria). We observed marked improvement in calibration accuracy following this protocol change and suggest this as an ongoing adaptation, regardless of pH/CO<sub>2</sub> control platform.

We developed a scalable, cost-effective, and modular system that accurately and precisely controls pH to allow for ocean acidification experiments in the case of the DA system. Modifications are also available to support combined parameter experiments due to the modular base system. This system will open up new avenues of investigation into one of the most significant threats to marine organisms, allowing more researchers to enter into this important area of study.

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