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Letter

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In Situ Detection of Species Relevant to the Carbon Cycle in Seawater with Submersible Potentiometric Probes

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ABSTRACT

We report on the development of a submersible probe for the simultaneous potentiometric detection of carbonate, calcium and pH in seawater. All-solid-state electrodes incorporating nanomaterials provide adequate response time (< 10 s), stability (drifts < 0.9 mV h^{-1}), reproducibility (calibration parameters deviation < 0.7%) and accuracy (deviation < 8% compared to reference techniques) for real time monitoring of seawater using a flow system. The functioning of the deployable prototype was checked in an outdoor mesocosm and during a long-term monitoring in Genoa Harbor. The electrodes were working properly during three weeks and the system demonstrated capability to autonomously operate with routines for repetitive measurements, data storage and management. In situ profiles observed in Genoa Harbor and Arcachon Bay were validated using on site and ex situ techniques. The validation of in situ detected carbonate is a challenge since both, re-equilibration of the sample with atmospheric CO_2 and the use of apparent thermodynamic constants for speciation calculations lead to some differences (< 20% of deviation). The submersible probe is a promising tool for obtaining rapid and trustable information about chemical levels in marine systems. Moreover, the fluidic approach allows for the integration of other ion sensors that may require sample pretreatment.

INTRODUCTION

The accurate measurement of the carbonate system in seawater is of urgent importance to study ocean acidification caused by the absorption of anthropogenically emitted CO_2.[1-4] Since the concentrations of the associated chemical species, CO_2, pH, HCO_3^- and CO_3^{2-}, are interconnected by thermodynamic constants, the carbonate system can in principle be described from the measurement of just any two species among the four.[5] Their choice depends on the performance of the available analytical techniques, including the possible implementation for in situ monitoring in the aquatic system of interest. Additionally, the quantification of dissolved calcium is related to the carbon cycle as it is involved in carbonate precipitation/dissolution processes and its monitoring may contribute to a more complete description of the marine system.[6, 7]

With pH values frequently measured in seawater using deployable glass electrodes, HCO_3^- and CO_3^{2-} are commonly calculated from total inorganic carbon or total alkalinity together with the pH after sampling, and CO_2 is detected as pCO_2 using different submersible probes that often require CO_2 to be in the gas phase.[8, 9] pCO_2 measurement techniques for in situ observations has been reviewed recently, discussing advantages and disadvantages of the existing approaches as well as the most promising concepts.[10] Accordingly, (i) non-dispersive infrared spectroscopy NDIR pCO_2 sensors constitute the most used technique for in situ measurements but frequent recalibrations are needed; (ii) CO_2 sensors based on Severinghaus principle exhibit reduced sensitivity, slow response time and an unstable signal at environmental pH (ca. 8.0), therefore their in situ application is not advisable;[11-13] (iii) pCO_2 optodes are still in the early stages of development, exhibiting slow response times, salinity dependence and poor stability, which must be improved upon before implementation into submersible probes.

An in situ operation of the techniques for carbonate species analysis is especially significant. They provide much higher spatial and temporal profiles compared to centralized approaches.[14] Moreover, the manipulation of the sample during sampling, transport and measuring procedures may dramatically modify its integrity by re-equilibration of the aquatic sample with an atmospheric CO_2 level that is very different from the in situ value and thus providing biased results.[5, 15-17] To avoid this, the samples must be kept out of air contact during manipulation and analysis, which is very difficult. Consequently, the most robust solution would involve the measurement in situ. Unfortunately, not all analytical techniques and required sample pretreatment steps are suitable for this goal.

An alternative approach is the use of all-solid-state ion-selective electrodes, which offer promising characteristics for in situ implementation,[18, 19] for the detection of either HCO_3^- or CO_3^{2-}. While determination of HCO_3^- in marine systems is not achievable so far by direct potentiometry owing to the high hydrophilicity of the anion and the lack of selective ionophores,[20] carbonate-selective electrodes based on N,N-dioctyl-3α,12α-bis(4-trifluoroacetylbenzoyloxy)-5β-cholan-24-amide have been satisfactorily applied for carbonate detection in freshwater and buffered marine samples,[21-23] and are promising for seawater analysis. Even if in situ detection is achieved, the validation of the sensors is challenging. Any reference technique requires sampling and/or indirect calculation of the species concentration using the abovementioned thermodynamic constants. The latter are not universally accepted as their apparent values strongly depend on environmental factors (i.e. salinity and temperature) and are therefore a possible source of error.[24-27]

We present here a new submersible potentiometric probe for the long-term real-time detection of pH, carbonate and calcium in seawater. A potentiometric flow cell based on miniaturized all-solid-state sensors is developed for this purpose. The flow cell is incorporated into a deployable device offering autonomous operation and the compensation of temporal effects in the electrode responses by intermittent single point calibration steps. The system was deployed in Mediterranean and Atlantic coastal waters near Genoa (Italy) and Arcachon (France), respectively. The probes were validated with traditional sampling techniques.

EXPERIMENTAL SECTION

Preparation of membrane-based ion-selective electrodes. Commercial glassy carbon macroelectrodes (GC-electrode-tip, 6.1204.300, Metrohm) and miniaturized hand-made glassy carbon electrodes (see Supporting Information and Figure S1a for the fabrication) were modified with functionalized multi-walled carbon nanotubes (f-MWCNTs) in analogy to earlier work.[22] Briefly, a film of the nanomaterial was deposited on top of each electrode by drop casting 8 × 20 µL (for macroelectrodes) or 5 × 5 µL (for miniaturized electrodes) of a f-MWCNTs solution in THF (1 mg mL^{-1}), allowing each layer to dry for 10 min before depositing the next layer. Then, the corresponding membrane cocktail (see Supporting Information) was drop cast on the top of the f-MWCNTs film (5 × 50 µL or 4 × 10 µL respectively), allowing each layer to be dried for 20 min. Finally, the electrodes were conditioned overnight (~12 hours) as detailed in Supporting Information. Once the sensors are ready they are implemented into the flow cell and finally into the submersible device.

Submersible device for in situ potentiometric measurements in seawater. Figure 1a shows the flow cell developed for the in situ potentiometric measurements. It consists of a cubic acrylic block (25 × 25 × 25 mm) incorporating single drilled holes: two on opposite sides for the inlet and outlet, one for a reference electrode fabricated in house[28] and other three holes for miniaturized potentiometric electrodes. In this manner, the inlet, outlet and the electrodes are concentrically placed around an inner chamber of the cell (20 µL volume) in which the sample flows, see Figure 1b. Figure 1c shows the fluidics of the submersible device, which uses a two-position valve (supplied by Idronaut) that allows the detection of either a calibration solution (pumped in from an external bag) or the seawater sample. This detection part is placed into a water- and pressure-proof cylindrical housing and is connected to external fluidics driven by a submersible peristaltic pump (see Supporting Information and Figures S1b-d). When the pump starts (flow rate of 200 µL $min^{-}1$) with the valve in the “normally close” position (NC), the “in situ calibration solution” (filtered seawater from the place where the deployment is planned) flows from the external bag to the valve and to the acrylic detection cell, and is subsequently discharged to the outside environment. In this way, the potentials of pH, carbonate and calcium electrodes are measured in flow mode in the calibration solution. If the valve is in the “normally open” position (NO), the cell is filled with filtered seawater from the aquatic system. During field deployment, the device is anchored onto a titanium cage containing the pump and other modules such as a CTD multiparameter probe (see Figure 1d). A detailed description of the measurement protocol, temperature and drift compensation as well as concentrations calculation is given in Supporting Information. Briefly, the electrodes are individually calibrated before the deployment using four standards solutions for each analyte. The slope (s) at environmental temperature (T_1) and standard potential (E^0) of each electrode is calculated. Then the potential of the three electrodes is measured in the “in situ calibration solution” ($E_1^{pH}, E_1^{Carb}, E_1^{Ca}$) in order to correct for the electrodes drifts. Thereafter the device is deployed and cyclic measurements (for instance every hour) of first the “in situ calibration solution” and then the seawater from the aquatic system are accomplished. The slope of each electrode is recalculated (s') for every measurement according to the water temperature (T_2, measured by the CTD) using equation 1. The standard potential is also recalculated ($E^{0\prime}$) and therefore electrodes drifts are compensated for every measurement using the recorded potentials on the calibration solution using equation 2.

$$s' = s\frac{T_1}{T_2} \tag{1}$$

$$E^{0\prime} = E_2 - \left[\frac{T_2}{T_1} \cdot (E_1 - E^0)\right] \tag{2}$$

Finally, pH, carbonate and calcium in situ concentrations are calculated using the corrected calibration graphs (with s' and $E^{0\prime}$) on the basis of potentiometry using the Nernst equation (eq. 3):

$$E = E^{0\prime} + s' \cdot \log\big(a_I(aq)\big) \tag{3}$$

where the slope is equivalent to [2.303·RT/zF] (R=gas constant, T=temperature, z=charge of the ion and F=Faraday constant).

RESULTS AND DISCUSSION

Characterization of all-solid-state potentiometric sensors for pH, carbonate and calcium detection in seawater.

The analytical performances of potentiometric macroelectrodes for pH, carbonate and calcium were assessed in 600 mM NaCl background and at pH 8.1 (for carbonate and calcium) to mimic environmental conditions in seawater. All-solid-state ion-selective electrodes (ISEs) based on carbon nanotubes were selected since they have already demonstrated to provide robust detection of ions in environmental water.[22,28-30] Calibration graphs observed for pH, carbonate and calcium (Figure S2) followed the common Nernstian behavior described for potentiometric sensors.[31] Importantly, the linear response ranges include the expected levels for the three species in seawater (from 7.5 to 8.5 for pH, about 10^{-4} M for carbonate and 10^{-2} M for calcium),[18] suggesting that the electrodes can be used directly for seawater analysis without any pretreatment, with a response time lower than 10 s. Other response characteristics, i.e. between-day reproducibility of the calibration parameters (Table S1), electrode drift (Table S2) and selectivity (Figure S3), were additionally evaluated to assure reliability of the calculation of unknown concentrations and suitability of the sensors for long-term measurements in seawater (see Supporting Information for more details). Although all the sensors demonstrated adequate performances in view of field measurements, even no influence of interfering ions in the potentiometric response (Figure S3), it is desired to implement simple corrections of the calibration parameters (slope and standard potential) to compensate for temporal variations. These are expected owing to environmental factors and/or (bio)fouling, that may provide biased results when measuring in situ. This may be performed by recalculating the electrode slopes according to observed variations of seawater temperature and repeatedly measuring the electrode potential in a calibration solution during deployment to correct the standard potential of each electrode.

The analytical performances of miniaturized hand-made glassy carbon electrodes (3-fold reduced size) that are physically more suitable for the coupling to the submersible device were found to be similar to those of macroelectrodes (see Tables S1 and S2 for a deeper comparison). The analysis of pH, carbonate and calcium was accomplished in different seawater samples using the macroelectrodes in batch mode, the miniaturized electrodes in flow mode (using the developed flow cell) as well as reference techniques (i.e. pH-meter, CO_2 probe and atomic absorption spectroscopy respectively) in order to validate the sensors (see Tables S3 and S4). A good agreement was found using the different techniques (deviations < 8%), confirming the ability of the designed potentiometric cell for seawater analysis.

Implementation and in situ functioning of the submersible potentiometric probes.

The next step was the physical implementation of the flow potentiometric cell into the submersible device, which is based on a fluidic system allowing the detection of either a calibration solution or seawater by the sensors (Figure 1c). The system was designed to operate automatically by controlling the fluidics (submersible pump), potentiometric outputs, data recording and managing with routines stored on board. For field deployment the module was mounted in a titanium cage together with the pump and other modules (CTD multiparameter probe), see Figure 1d.

To evaluate the in situ functioning of the system, this was deployed for 58 h in an outdoor mesocosm in Plentzia (Spain) monitoring pH, carbonate and calcium with the potentiometric probes simultaneously with the CTD probe. The experimental protocol for in situ measurements is described in the Supporting Information (including the correction of the calibration parameters). As shown in Figure S4, the potentiometric probes were properly measuring during this entire time. The electrodes displayed very small drifts during the three minutes of potentiometric measurements in both the calibration solution and the seawater ($< 5\ \mu V\ min^{-1}$). In addition, pH data were validated with the CTD measurements showing uncertainties < 0.15% in all the cases. Later, the lifetime of the sensors during real-time measurements in seawater was evaluated during a long-term experiment conducted in the Genoa harbor (Italy) deploying the system at 4.2 m depth from a fixed platform (CNR Station). The electrodes were working satisfactorily for three weeks (Figure S5) displaying absolute uncertainties of $\Delta pH<0.001$pH units, $\Delta c_{Carb}<0.002$ mM and $\Delta c_{Ca}<0.02$ mM (calculated from the standard deviation of the recorded potentials). For a longer period of field work, it is currently advisable to replace the sensors to avoid biased results due to the deterioration of the membranes and reference electrodes. The pH measurements were again validated compared to the CTD data (uncertainties < 0.12% without considering the first 6 days of the monitoring).

Medium-term deployment in Genoa Harbor (Italy).

In situ operation of the sensors was validated during the first 10 h of a 167-h monitoring period in the Genoa Harbor by sampling and measuring on site and/or ex situ using the same procedures as in the laboratory. Linear correlations were obtained in all the cases (Figures S6-S8) with uncertainties lower than 0.8%, 22% and 1.6% for pH, carbonate and calcium concentrations respectively. While any possible bias could be likely associated to the sampling procedure, observed uncertainties are acceptable for pH and calcium detection although carbonate validation requires special attention. The values observed for on site detection using macroelectrodes and the CO_2 sensor were found to be always higher than those measured in situ (uncertainties of 10-15% and 15-22% respectively), likely due to equilibration of the sample with atmospheric CO_2. On the other hand, the use of the CO_2 probe implies a series of calculations to obtain the carbonate concentration that are error-prone as well. This behavior was previously also found by others who aimed to validate measurements of the carbonate system and reported deviations close to 50% due to the combination of all the mentioned factors.[15,17]

Temporal profiles observed for the whole 167-h period are shown in Figure 2, exhibiting minimum in carbonate and calcium levels that are reached during the daylight hours and coinciding with temperature maxima. These day/night cycles are in accordance with the natural fluctuations of the carbon cycle, in which the absence of light causes CO_2 production by the organisms. As natural activity in a harbor is reduced compared with the open sea,[32] note that average dissolved O_2 (6.4 ± 0.3 ppm) corresponds to 83.7 ± 3.3% oxygen saturation, the carbonate increase during the night may be also related to an increase in the CO_2 levels with decreasing temperature.[33] In addition, observed increases/decreases in carbonate levels are associated with calcium concentrations following exactly the same trends. The average concentrations of carbonate and calcium, 0.22 ± 0.20 mmol L^{-1} and 11.82 ± 0.61 mmol L^{-1} respectively, are within the expected levels in seawater[18] and the molar percentage of carbonate with respect to calcium (~2%) coincides with the carbonate speciation at the environmental pH as calcium is mainly associated with carbonate precipitation/dissolution processes.[34, 35] The variations observed in the pH profile are within ±0.07 pH units indicating no drastic changes. A good agreement with the results from the CTD is also found.

In situ monitoring in Arcachon Bay (France).

The submersible potentiometric probes were also tested in Arcachon Bay (France) by means of two different monitoring approaches: (i) a day/night cycle in the harbor of 14 h duration and (ii) the analysis of different locations along the freshwater effluent of the Eyre River in the bay during high tide conditions. In both cases the deployment was performed from a boat using a small crane. Importantly, the southwest corner of the bay is open to the sea, generating cycles of high and low tide thus leading to salinity cycles, which involves changes in the levels of key chemical species such as nutrients.[36] For this reason, real-time monitoring of this area is significant for understanding (bio)geochemical processes locally occurring in the bay.

Observed profiles during the 14 h monitoring are presented in Figure 3. With regard to the pH measurements, no drastic changes were observed while, as expected, variations of carbonate and calcium levels are related to the tidal cycles. Minimal concentrations coincide with high tide conditions, while the maximum ones are found at low tides as a consequence of larger freshwater effluent loading of carbonate species better known as “tidal flushing” effect.[37] Carbonate and calcium levels are slightly lower during the daylight hours and the carbonate-to-calcium ratio is in agreement with the speciation. Moreover, in situ degree of seawater saturation with respect to calcite and aragonite may be directly calculated from calcium and carbonate concentrations according to equation 3:

$$\Omega = \frac{c_{Ca} \cdot c_{Carb}}{k_{sp}} \tag{3}$$

where the apparent solubility product (k_{sp}) is referred to either calcite or aragonite. As observed in Figure S9, similar tidal trends were obtained and the calculated values range from 4.1 to 5.2 for calcite and from 2.8 to 3.5 for aragonite when k_{sp} values of $5.94 \cdot 10^{-7}$ and $8.76 \cdot 10^{-7}$ $mol^2\,L^{-2}$ (atmospheric pressure, T=25°C and salinity=34.5‰) are respectively used[38] (note that the calculated values may change because of temperature, pressure and salinity influence on the values of k_{sp} and, as far as we know, there are no universal equations to calculate these values depending on the environmental conditions).[39] To the best of our knowledge, this is the first time that direct in situ measurements of calcium and carbonate are accomplished in Arcachon Bay.

For the second monitoring, Table 1 shows the results of a physical displacement to the west along the bay while the salinity was not drastically fluctuating. While no drastic changes were observed for pH, carbonate and calcium levels increased with westward displacement in agreement with a higher freshwater content. Validation of the observed concentrations shows uncertainties lower than 0.5%, 10% and 0.3 for pH, carbonate and calcium respectively, confirming again the suitability of the developed sensors for in situ seawater analysis.

In summary, we have developed submersible potentiometric probes to detect pH, carbonate and calcium in seawater in an autonomous manner with rapid data acquisition. The environmental application of the system is demonstrated in different aquatic areas of significance. The flow mode adopted for the measurements allows the correction of electrode drifts and makes it possible to integrate further inline pretreatments as needed for the potentiometric detection of other relevant ions.[28, 29]

ASSOCIATED CONTENT

Supporting Information.

Supporting Information available: Experimental section with materials, reagents, additional techniques, composition of membrane cocktails, fabrication of the miniaturized electrodes (Figure S1), preparation of the reference electrode, description of the submersible housing, experimental protocol for in situ measurements and data treatment. Analytical performances of the sensors (Tables S1 and S2). Validation of the sensors in the laboratory (Tables S3 and S4). Calibration graphs (Figure S2). Response to interfering ions (Figure S3). Monitoring in Plentzia (Figure S4). Long-term monitoring in Genoa Harbor (Figure S5). Validation of in situ measurements in Genoa Harbor (Figures S6-S8). Map of the studied locations in Arcachon bay (Figure S9).

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Figures Captions

Figure 1. Images of (a) the designed potentiometric flow cell and (b) the assembled flow cell incorporating the electrodes, inlet and outlet. (c) Developed fluidics for the in situ potentiometric measurements. The pump draws either filtered seawater or the calibration solution according to the valve position (NO=normally open, NC=normally close), whose outlet (OUT) is connected to the potentiometric flow cell. (d) The submersible probe is implemented into a titanium cage containing other modules as well as the pump. 1: electronics, 2: potentiometric cell, 3: bag for the calibration, 4: pump, 5: CTD multiparameter probe.

Figure 2. In situ profiles obtained for (a) temperature (CTD), (b) pH (CTD and developed electrodes), (c) carbonate and (d) calcium during a 167-h deployment (from April 3th, 2017 at 07:00 to April 10th, 2017 at 12:00) in the CNR Station at the Genoa Harbor (Italy). Note that additional sampling was performed for validation during the first 10 hours. Average salinity = 37.95 PSU. Depth of the deployment = 4.2 m. Dissolved O_2 = 6.4 ± 0.3 ppm corresponding to 83.7 ± 3.3 % for oxygen saturation within the monitoring. Light hours are indicated with gray squares.[40]

Figure 3. In situ profiles obtained for (a) pH (CTD and developed electrodes), (b) carbonate and (c) calcium during a 16-h deployment (from May 17th, 2017 at 17:00 to May 18th, 2017 at 09:00) in the Arcachon Harbor. Depth of the deployment = 1.5 m. The local time for high and low tides (HT and LT) are determined according to the tidal record at Jetée d'Eyrac (44°40'N 1°10'W) and considering the temporal evolution of the seawater level in the Arcachon basin.[41,42] Light hours are indicated with gray squares.[43]

Tables

Table 1. Results obtained in different locations of Arcachon Bay (France) on May 18th, 2017 using the submersible device incorporating the multiparameter CTD (T, salinity, dissolved O_2 and pH) together with the developed potentiometric probes for pH, calcium and carbonate. Additional ex situ and on site measurements carried out for validation are included. (High tide ~ 11:20 am and next low tide ~ 17:00 pm).

					pH		Ca^{2+} (mM)		CO_3^{2-} (mM)		
location[a]	hour	T (°C)	PSU	DOXY[b]	CTD	in Situ[c]	AAS	in Situ[c]	Sev[d]	macro[e]	in Situ[c]
AHB	9:03	17.8	32.1	6.92	7.92	7.90	10.82	10.81	0.31	0.30	0.28
PM	10:30	17.9	33.1	7.66	8.00	7.98	10.97	10.98	0.32	0.32	0.30
	10:50							11.01			0.31
LH	11:45	17.8	31.2	7.77	7.92	7.88	11.10	11.09	0.36	0.35	0.33
	12:05							11.10			0.33
CG	12:50	17.8	31.1	7.81	7.90	7.88	11.23	11.20	0.38	0.38	0.37
	13:11							11.21			0.36
	13:36							11.19			0.36

[a] AHB: Arcachon Harbor (44°39.652'N 1°09.050'W); PM: Pointe de la Matelle (44°39.708'N 1°07.711'W); LH: La Hume (44°39.750'N 1°06.709'W); CG: Chenal de Gujan (44°39.409'N 1°05.114'W).

[b] ppm

[c] In Situ measurements using the developed submersible device based on ISEs for pH, calcium and carbonate.

[d] Calculated from TCO_2 (total CO_2) obtained from on site potentiometric measurements using a Severinghaus probe.

[e] Obtained on site by using macroelectrodes analogous to those used into the submersible probe.

TOC Art

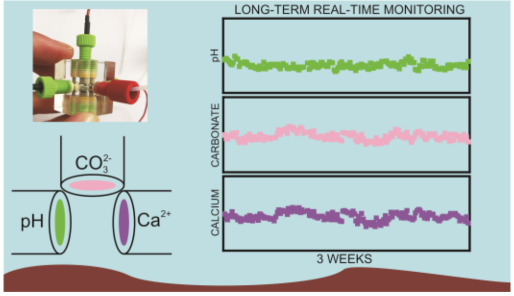

LONG-TERM REAL-TIME MONITORING
pH
CARBONATE
CALCIUM
3 WEEKS
CO_3^{2-}
pH
Ca^{2+}

Figure 1

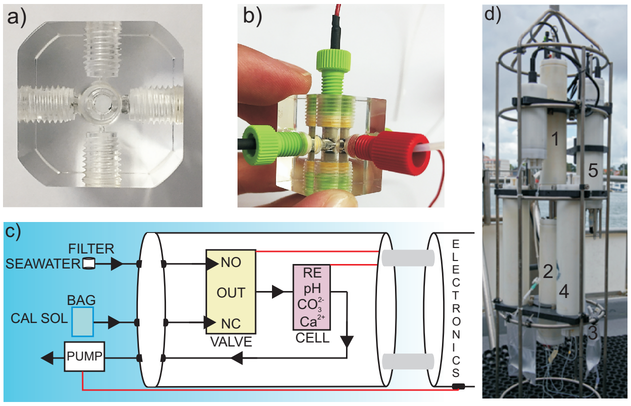

a)
b)
c)
d)
FILTER
SEAWATER
BAG
CAL SOL
PUMP
NO
OUT
NC
VALVE
RE
pH
CO$_3^{2-}$
Ca^{2+}
CELL
ELECTRONICS
1
5
2
4
3

Figure 2

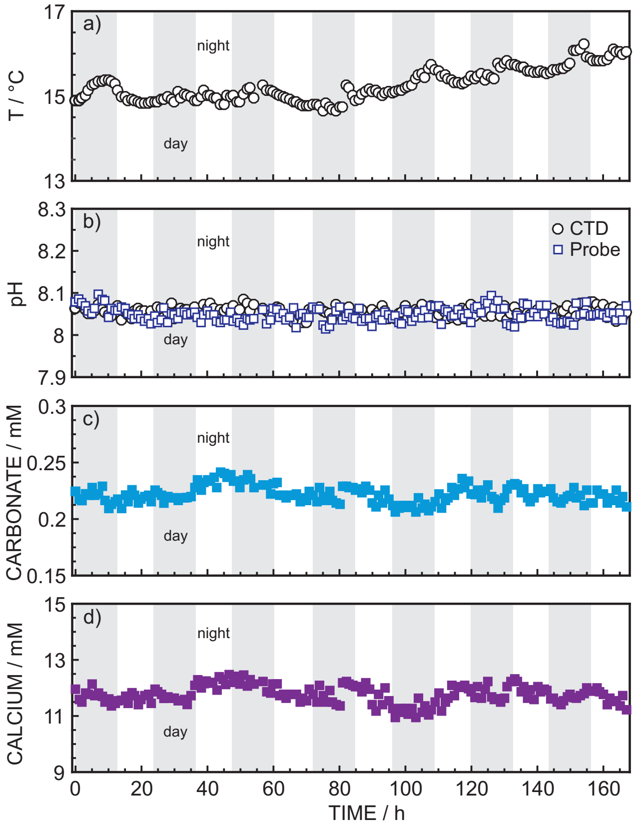
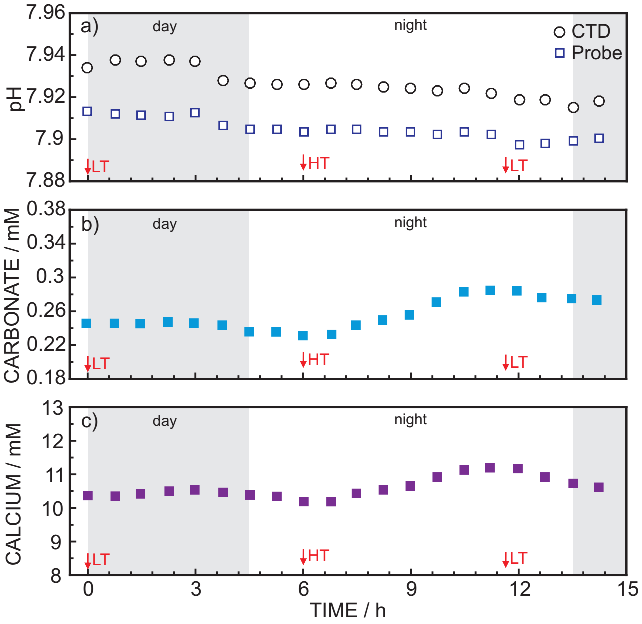

a)
night
day
T / °C
17
15
13
b)
night
day
○ CTD
□ Probe
pH
8.3
8.2
8.1
8
7.9
c)
night
day
CARBONATE / mM
0.3
0.25
0.2
0.15
d)
night
day
CALCIUM / mM
15
13
11
9
0
20
40
60
80
100
120
140
160
TIME / h

Figure 3


a)
day
night
○ CTD
□ Probe
pH
7.96
7.94
7.92
7.9
7.88
↓LT
↓HT
↓LT
b)
day
night
CARBONATE / mM
0.38
0.34
0.3
0.26
0.22
0.18
↓LT
↓HT
↓LT
c)
day
night
CALCIUM / mM
13
12
11
10
9
8
↓LT
↓HT
↓LT
0
3
6
9
12
15
TIME / h