Over the past two centuries, the accumulation of fossil-fuel CO₂ in the upper ocean has increased the gaseous CO₂ concentration but decreased pH and the carbonate ion concentration (Feely et al. 2004; Sabine et al. 2004). Consequently, surface water pH is now about 0.1 units lower than it was during the pre-industrial era, and it is predicted to fall by an additional 0.3 units by 2100 (Caldeira et al. 2003). Such a reduction in pH will lead to lowering of the saturation state of seawater with respect to biogenic calcite and aragonite (Feely et al. 2002, 2004; Chung et al. 2003, 2004), and a reduction in the rate of calcium carbonate production in corals (Orr et al. 2005). Changes in ocean carbonate chemistry influence not only sensitive marine ecosystems (e.g., calcifying organisms), but also other interacting components (e.g., preys, predators, and competitors) via changes in nutrient use of phytoplankton and growth rate of heterotrophic organisms. The transfer of these effects through the ecosystem could be either repressed or reinforced, depending on the occurrence of positive or negative feedback loops. Therefore, the mechanisms tending to repress or reinforce the effects of changes in ocean carbonate chemistry should urgently be identified to enable scenarios of future impacts on ecosystem function and biogeochemical cycles to be developed. To gain an integrated understanding of the sensitivity of marine biota to anthropogenic perturbations, there is a particular need for manipulative experiments at the community and ecosystem levels. Such experiments can be undertaken in large enclosures or the open ocean, but enclosed experimental systems enable easy manipulation of experimental conditions and are amenable to a greater degree of replication and repeatability than open ocean experiments (Kemp et al. 1980; Kareiva 1989).

The response of the marine ecosystem to fossil-fuel CO₂ and associated biogeochemical feedback processes has been investigated in a series of mesocosm-based experiments (Engel et al. 2004; Rochelle-Newall et al. 2004; Engel et al. 2005; Grossart et al. 2006; Kim et al. 2006). Two major mesocosm facilities used in these experiments have proved useful for in situ pCO₂ manipulation experiments. Of these, the
European Union Large Scale Facility (LSF) in Bergen, Norway, was used in most of mesocosm studies noted above. It consists of nine polyethylene gas-tight enclosures (5 m in height and 2 m in diameter, a total volume of ~11 m³). Air with the target pCO₂ concentration, produced by mixing ambient air with high pCO₂ air or CO₂-free air, is introduced into the enclosure headspace to maintain the target concentration, and into an air-lifting device for mixing with seawater (Williams and Egge 1998; Engel et al. 2005).

The design and performance of the above mesocosm facility have yet to be described in detail and assessed. In the present study, we evaluated the performance, over a wide range of experimental conditions, of a pCO₂ regulation unit and a bubble-mediated seawater mixer, both of which are key components of our mesocosm facility. We document the optimal operational settings that ensure generation of the target pCO₂ concentrations in the headspace and enclosure seawater and show that the experimental setup enhances the homogeneity of seawater and causes minimal disturbance of planktonic organisms.

**Materials and methods**

**Description of the components of the CO₂ mesocosm facility**—Our CO₂ mesocosm facility is located at Jangmok (34.6°N and 128.5°E) on the southern coast of Korea. The facility consists of a floating raft, nine impermeable enclosures with transparent caps, nine pCO₂ regulation units, and nine bubble-mediated seawater mixers. Fig. 1 shows the configuration of the major components of the facility.

**Floating raft**—The raft (approximately 19 m long and 3 m wide) is constructed from steel frames and mounted on rubber floats anchored to the sea floor approximately 50 m seaward of a pier. The nine enclosures are moored to the floating raft and aligned perpendicular to the sun's pathway to ensure that all receive approximately the same intensity of solar radiation. The average water depth beneath the raft is 5 m on the ebb tide and 9 m on the flood tide.

**Impermeable enclosures with transparent caps**—The enclosures are cylindrical (3 m in height, 1 m in diameter) and made of polyethylene. Approximately the top 1 m of each enclosure is above the water surface to prevent the accidental incursion of seawater due to wave action. The top of each enclosure is covered with a hemisphere-shaped acryl cap (approximately 1.2 m in diameter and 0.4 m in height) that is transparent to solar radiation. The gap between the enclosure and the cap is less than 1 cm.

**pCO₂ concentration-regulation units**—The pCO₂ regulation units were designed to generate air with a wide range of pCO₂ concentrations and to dispense the air to enclosure headspaces and seawater. The pCO₂ target values were 280, 390, and 950 μatm, representing the pre-industrial, present-day, and predicted year 2100 atmospheres, respectively. The pCO₂ value of 950 μatm for the year 2100 was based on model projections using one of the Intergovernmental Panel on Climate Change’s emission scenarios (A1FI; Houghton et al. 2001). Each of the target pCO₂ concentration air masses was produced by three replicate pCO₂ regulation units. All nine pCO₂ regulation units were separately encased in stainless steel panels (36 cm wide, 15 cm long, and 40 cm high), the surfaces of which were anodized to prevent rusting associated with the deposition of sea salts. The main components of the pCO₂ regulation units (Fig. 2) are described in details in the following sections.

**Air pumps**—A gas flow sufficient to flush the 1000 L enclosure headspace, maintain the target pCO₂ concentration in the headspace, and maintain flow into the seawater mixer requires a considerable gas flow rate. A further consideration is the air pump emitting pressure. For normal operation of a static gas mixer (see Fig. 3), emitting pressures greater than 1 kPa are required. Oil-less rocking piston air pumps (75R645–P101–H302X; GAST, USA) were chosen to meet these requirements. Each pump is 24 cm wide × 9 cm long × 19 cm high, weighs approximately 8 kg, and can generate an air flow rate of approximately 140 L min⁻¹ and a maximum pressure of 2 kPa in open flow mode. In the mesocosm configuration, the pumps produce an air-flow rate of 50 L min⁻¹ and air pressure of approximately 1 kPa. These reductions in efficiency are probably caused by resistance in the gas lines and controlling valves installed along the lines.

**Water vapor removal units**—We cool the hot air (>80°C) emitted by the air pumps by forcing it through a submerged stainless steel fiber-coated Teflon line. The cooled air then passes through an air filter (AF320-10; Parker) for collection of condensed water in a drain pot. To enable further drying, the
Air is passed through silica gel (3 mm particle size) in an acryl tube (8 cm diameter and 40 cm long) with screw caps at both ends. The silica gel is replaced approximately every 10 h when used under the standard air-flow rate. Both an air filter and a desiccant tube are installed upstream of the pCO₂ regulation unit to avoid malfunction of the unit due to condensation.

Gas-mixing units—Each gas-mixing unit consists of a ball flow meter (060801-227, 060710-161, 060801-222; KOFLOC, Japan), a mass flow controller (3660; KOFLOC, Japan), and a static gas mixer (T6-15-21; Noritake, Japan). The mass flow controller accurately delivers ultra-pure (99.999%) CO₂ at varying rates (from 10 to 320 mL min⁻¹) into the gas mixer where the CO₂ is thoroughly mixed with ambient air at a high flow rate controlled by a ball flow meter. The real-time flow rate of the ultra-pure CO₂ stream can be read from a readout box.

The static gas mixer is a key component of these gas-mixing devices and is housed in a thin-walled tube for bite-type fitting (Fig. 2). The mixers have an internal diameter of 11 mm and are 26 cm in length. Each mixer contains 15 rectangular plates (1.5 times their internal diameter) twisted at 180° and aligned alternately at an angle of 90°. Both the ultra-pure CO₂ and ambient air streams simultaneously flow into a T-shaped connector within which both gases are roughly mixed. For more thorough mixing, the gas mixture subsequently flows into the first plate within which the gas mixture is bisected. As the bisected gas streams enter the second plate, they merge and the stream is then bisected again into two new gas streams (Fig. 3). By repeating this process 15 times, the small amount of added ultra-pure CO₂ is mixed thoroughly and rapidly with the ambient air. According to the direction of the twist, the gas streams are rotated either toward the pipe wall or to the center. This configuration facilitates thorough mixing of the gases by creating a strong flow-reversal motion. This gas mixer enables generation of target pCO₂ levels to be achieved that are either higher or lower than the ambient level.

**Fig. 2.** Diagrams of two different pCO₂ regulation units. (A) Unit for producing pCO₂ concentrations higher than the ambient value. (B) Unit for producing pCO₂ concentrations lower than the ambient value. The dashed and thin solid lines in (A) and (B) indicate pathways of ambient air and pure CO₂ (or CO₂-free air), whereas the thick solid lines indicate pathways of target pCO₂ air.

**Fig. 3.** A conceptual diagram of gas mixing within a static gas mixer. Blue and red arrows indicate streams of ambient air and ultra-pure CO₂, respectively. Purple arrows indicate bisection and mixing of the gases by the plates.
Generating $pCO_2$ levels that exceed ambient concentrations—
The $pCO_2$ gas regulation unit shown in Fig. 2A produces air with $pCO_2$ levels higher than the ambient concentration (approximately 390 $\mu$atm). Simultaneous entry of ambient air and ultra-pure CO$_2$ to a static mixer at flow rates of 50 L min$^{-1}$ and 40 mL min$^{-1}$, respectively, generates air with a $pCO_2$ value of approximately 950 $\mu$atm. A minimum tank pressure of 2.5 kPa in the ultra-pure CO$_2$ tank is needed to generate a flow rate of 40 mL min$^{-1}$.

Generating $pCO_2$ levels below ambient concentrations—The $pCO_2$ regulation unit shown in Fig. 2B produces air with $pCO_2$ levels lower than the ambient concentration by mixing ambient air and CO$_2$-free air. To produce CO$_2$-free air the ambient air stream is passed at a rate of approximately 20 L min$^{-1}$ through a desiccant tube (8 cm diameter, 40 cm long) containing 1.5 kg of Na$_2$CO$_3$ platelet crystals. The CO$_2$-free and ambient air streams are directed concurrently into the gas mixer at equivalent flow rates of approximately 20 L min$^{-1}$.

Generating ambient $pCO_2$ levels—The chosen pump directly delivers ambient air into the headspaces of each enclosure, and the seawater mixer housed therein.

Seawater mixers—Previous studies used air lifting (Engel et al. 2005) and mechanical stirring (Alldredge et al. 1995) to mix seawater within enclosures. In our mesocosm system, we use two acryl pillars installed in each enclosure to gently aerate the seawater with air at the target $pCO_2$ concentration to ensure the homogeneous distribution of phytoplankton and solutes. Each seawater mixer consists of two pillars (15 cm diameter and 1.8 m high) 50 cm apart and attached to individual vertical poles for support (Fig. 4). Each mixing unit has two spare supporting poles for additional pillars. All these components are supported by polypropylene rings (approximately 80 cm diameter), one each at the top, in the middle, and at the bottom (Fig. 4). Bubbles in each pillar are generated by a bubble stone (11 cm diameter; S104-B; SUDO, Japan) located approximately 50 cm above the bottom of the pillar. As fine bubbles from the bubble stone push seawater from the lower part of the enclosure to the surface, outside seawater is introduced into the pillar through holes just below the bubble stone. Consequently, incoming seawater is continuously transferred to the surface by rising bubbles. This mixing scheme generates a convective flow of seawater within the enclosure that enhances the homogeneity of the seawater in terms of phytoplankton cell density and solute concentration.

Sequence of the mesocosm setup—Following introduction of seawater into the enclosures, the target $pCO_2$ concentration in the enclosed seawater is usually attained within a day using the $pCO_2$ regulation units. After adjusting the seawater $pCO_2$, target concentration $pCO_2$ air mass is pumped only into the enclosure headspace for the duration of the experiment. For 20 min per day, a small fraction (approximately 0.5 L min$^{-1}$) of this air is diverted into the seawater mixer, while the major fraction (approximately 49.5 L min$^{-1}$) continues to flow into the enclosure headspace. Immediately after 20 min, seawater aeration is completed the enclosures are sampled. This dichotomous gas flow scheme significantly reduces seawater sampling bias while maintaining the target $pCO_2$ value in the headspace.

Assessment

Generation of target $pCO_2$ concentrations using the $pCO_2$ concentration regulation units—The performance of our $pCO_2$
regulation unit was assessed by measuring the degree to which resulting pCO₂ concentrations coincided with target pCO₂ levels (280 to 950 μatm). In particular, the resulting pCO₂ levels were sensitive to the ratio of ultra-pure CO₂ and ambient air entering the gas mixers: the higher the gas mixing ratios the higher the resulting pCO₂ concentrations. For example, we varied the flow rate of ultra-pure CO₂ between 10 and 120 mL min⁻¹ while maintaining a constant flow rate (50 L min⁻¹) of ambient air. The pCO₂ concentrations of the resulting air were measured using an infrared analyzer (Li–Cor 820), and showed a linear trend with increasing release rates of ultra-pure CO₂ (Fig. 5). For the three pCO₂ regulation units tested, the increase in pCO₂ as a function of the rate of ultra-pure CO₂ added was nearly identical.

Regulation of target pCO₂ concentrations in enclosure headspaces—We continuously monitored the pCO₂ concentrations in the headspaces of two enclosures to assess how quickly the target pCO₂ value was reached and whether it could be maintained (Fig. 6). To maintain the target value, the enclosure headspaces were largely isolated from the outside atmosphere and were continuously flushed with 50 L min⁻¹ of air flow. Immediately following input of target pCO₂ air into the enclosure headspaces, we sampled air from approximately 20 cm above the air-water interface within the enclosures. The target pCO₂ concentration was achieved within 30 min of input of the target pCO₂ air and was maintained at 958 ± 10 μatm thereafter (Fig. 6). Under the wind speed condition (2–6 m s⁻¹) at which this experiment was undertaken, the target pCO₂ value remained approximately unchanged with only minor fluctuations. The tightly closed enclosure-cap system ensures a stable concentration of pCO₂ in the headspace. With the gas regulation unit in the described configuration, the lowest achievable pCO₂ concentration within the headspace was approximately 287 ± 5 μatm (Fig. 6), which is comparable to the pCO₂ value during the pre-industrial period.

Generation of target pCO₂ concentrations in enclosure seawater—Immediately after adding seawater to the enclosures, we bubbled pCO₂-free air into one set of three enclosures at a rate of approximately 0.5 L min⁻¹, to generate a target seawater pCO₂ level (280 μatm) below the ambient level. We also bubbled air with a pCO₂ level four times the target concentration into another set of three enclosures to achieve a seawater pCO₂ level of 4000 μatm (Fig. 7).
This enabled us to assess changes in seawater carbonate parameters in enclosures with target pCO2 levels of 280 μatm and 950 μatm. The bubbling rate was sufficiently gentle to cause minimal impact on the phytoplankton assemblage. Using this pCO2 manipulation method, seawater pCO2 levels in the enclosures attained the target concentrations within 24 h (Fig. 7, and the shaded bar in Fig. 8C). The slopes of the best-fit trends relating the aeration time and the pCO2 concentration in seawater samples differed significantly, depending on the pCO2 concentration in the injected air: 23.4 ($r^2 = 0.9794$) for 4000 μatm and 2.9 ($r^2 = 0.8484$) for 950 μatm. The seawater pCO2 concentrations were not measured directly because the protocol for using an infrared analyzer/equilibrator-based pCO2 system involves removal of phytoplankton from the seawater. Consequently, calculations of the seawater pCO2 (and pH) levels in the enclosures were based on measurements of total dissolved inorganic carbon (CT) and total alkalinity (AT) of the samples using the carbonic acid dissociation constants.

Fig. 8. Changes in (A) temperature, (B) salinity, (C) pCO2, (D) total dissolved inorganic carbon (CT), (E) total alkalinity (AT), and (F) pH in enclosure seawater over an 8-d period that includes the 1-d aeration period. The headspaces of the two enclosures were continuously filled (from Day 0 to Day 7) with pCO2 values of 280 μatm (open circles) or 950 μatm (filled triangles) pCO2. The shaded bars represent the 1-d aeration period during which either pCO2-free or 4000 μatm pCO2 air were bubbled into the enclosure seawater to adjust the seawater pCO2 to the target values of 280 μatm and 950 μatm, respectively. Error bars represent the standard deviations of the mean results of the replicate enclosures.
of Mehrbach et al. (1973) that were refitted in different functional forms by Dickson and Millero (1987). This set of thermodynamic constants has proved to be the most consistent with laboratory (Lee et al. 1996; Lueker et al. 2000; Mojica Prieto and Millero 2002; Millero et al. 2006) and field (Lee et al. 1997, 2000; Wanninkhof et al. 1999; Millero et al. 2002) measurements of carbon parameters over the oceanic ranges of temperature and salinity. Given the uncertainty (± 2 μmol kg–1) in CT and AT measurements, the predicted pCO2 and pH values based on CT and AT were accurate to ± 5 μatm and ± 0.005 units, respectively. To monitor changes in seawater pCO2 levels in the enclosures, it is recommended that measurements of CT and AT be performed approximately daily over the duration of mesocosm experiments.

The bubbling procedure changed the seawater carbonate chemistry without changing the seawater temperature or salinity. The temperature (7.4 ± 0.1°C) and salinity (33.0 ± 0.01) of seawater in the enclosures were approximately constant for the initial 24-h period (the shaded bars in Figs. 8A and 8B). The target pCO2 level determined the degree of change in pH and Cτ. For example, there was a 150 μmol kg–1 increase in Cτ concentration in the enclosure with a target pCO2 concentration of 950 μatm, whereas there was a 21 μmol kg–1 decrease in Cτ concentration in the enclosure with a target pCO2 concentration of 280 μatm (the shaded bar in Fig. 8D). As expected, Aτ remained unchanged with addition of air at the target pCO2 concentration (the shaded bar in Fig. 8E). The calculated pH decreased by approximately 0.44 units in enclosures with a target pCO2 concentration of 950 μatm, but increased slightly (0.04 units) in enclosures with a target pCO2 concentration of 250 μatm (the shaded bar in Fig. 8F).

We also monitored changes in seawater carbonate parameters over the 7-d period after seawater pCO2 in the two enclosures reached the target levels (Day 0 through Day 7, Fig. 8). Seawater pCO2 and Cτ in the enclosures gradually decreased due to biological activity, which also had the effect of increasing seawater pH. The Aτ in seawater increased slightly (5–6 μmol kg–1) in the two treatments due to evaporation-induced salinity increase. The magnitudes of the reductions in pCO2 and Cτ observed during the experiment were lower than expected, due to the flux of CO2 from the mesocosm atmosphere.
Evaluation of the degree of homogeneity of seawater samples—
To determine the optimal aeration rate and time to minimize the standard deviation of the measured particulate organic carbon (POC) concentrations, we aerated the seawater at three different gas flow rates: 0.5, 1.0, and 1.5 L min⁻¹. We then took duplicate samples from each of the surface, middle, and lower (the depth at which the bubble stone was installed) parts of an enclosure at each of four different stages: prior to aeration, and at 20, 40, and 60 min after aeration was initiated. In the absence of seawater, mixing the POC concentrations were highest in samples taken from the lower parts of the enclosure (Fig. 9). This probably reflects the accumulation of organic matter by settlement.

Variations in POC concentration with depth was significantly reduced in samples taken 20 min after initiation of aeration (Fig. 10). In particular, the standard deviation from the average of the six POC concentrations decreased significantly, from 30% prior to aeration to less than 10% at 20 min after aeration was initiated, regardless of the aeration flow rates. The results of this experiment indicate that the degree of homogeneity of seawater samples was optimized within 20 min of aeration with an aeration flow rate of 0.5 L min⁻¹.

It is also important to note that the 20 min aeration process affected seawater pCO₂ concentrations to a minor degree; for example, for 950 μatm pCO₂ air the pCO₂ concentration in seawater increased by less than a few micro-atmospheres. This elevation in seawater pCO₂ was predicted from the equation relating aeration period and seawater pCO₂ concentration, shown in Fig. 7.

Evaluation of initial phytoplankton composition after filling the enclosures with seawater—After filling all enclosures with seawater, we assessed phytoplankton composition across the nine enclosures. The mean phytoplankton concentration (>10 μm) in the nine enclosures was 278,030 ± 84,020 cells L⁻¹. The relative abundances of the two major phytoplankton species Eutreptiella gymnastica and Skeletonema costatum were similar across the nine enclosures (Fig. 11). Their combined populations accounted for approximately 95% of the population in all but one enclosure (C1) (Fig. 11). Of the two main species, E. gymnastica was by far the major component, accounting for approximately 84% ± 7% when averaged over all nine enclosures.

Discussion
We report the design of a mesocosm facility that was established and thoroughly tested at Jangmok (34.6°N and 128.5°E), on the southern coast of Korea. The fully automated pCO₂-controlling system consistently and accurately delivered air to the headspaces and seawater in enclosures at pCO₂ concentrations covering the range from pre-industrial to projected year 2100 atmospheres. The seawater mixing device gently and thoroughly mixed seawater in the enclosures within 20 min of addition, and with minimal perturbation. Performance evaluations indicated that the mesocosm facility is suitable for in situ experiments that involve testing of the short-term effects (approximately 30 d) of pCO₂ on a marine ecosystem. The proposed duration of mesocosm experiments is sufficiently long to observe impacts of seawater pCO₂ concentration changes on relatively sensitive ecosystem processes, such as the calcification rates of calcifying organisms (Engel et al. 2005) and growth rates of diatom species (Kim et al. 2006). Other important
scientific questions that need to be addressed include the effects of seawater pCO2 concentration on carbon export production and shifts in phytoplankton species composition. Future studies should also focus on assessing the reliability (including the experimental duration) of mesocosm-based manipulative studies, in particular their adequacy for addressing these urgent scientific issues.

Although our mesocosm facility emulated the oceanic environment, the design of the system produces three artifacts. First, as with other mesocosm-based studies, these studies are inadequate for assessing the chronic effects of CO2 perturbations because of wall effects and deviations of the plankton community within the enclosures from the nearby natural system. Second, phytoplankton within the enclosures are unlikely to experience natural levels of physical turbulence that occur in the ocean. Third, experiments that exclude higher trophic levels provide no insight into the potential effects on phytoplankton growth of interactions between phytoplankton and zooplankton, of organisms of other trophic levels. However, mesocosm experiments could be performed with natural plankton assemblages, without the preferential removal of higher trophic levels.

Despite their limitations, in situ mesocosm pCO2 manipulation studies provide an effective tool for unravelling the effects of projected future forcing on natural aquatic ecosystems, and provide a link between in vitro experiments and field observations. As human-induced climatic change continues to alter marine environmental conditions, manipulative experiments ranging from the community level to the scale of entire ecosystems will become increasingly relevant.

Comments and recommendations

Many minor factors can collectively affect the success of CO2 manipulation experiments using a mesocosm facility. Here, we make recommendations regarding three major operational factors that are critical in obtaining significant and reproducible results in mesocosm experiments.

The first is ensuring that all enclosures are leak-proof prior to setting up a mesocosm facility. To check for leaks, we lifted each enclosure using a crane and filled it with approximately 250 L tap water. We then laid the enclosure on a dry floor and, paying particular attention to joins, rolled it to detect leaks. If found, these were carefully sealed using silicon glue. The leak test should be repeated for each enclosure until all leaks are eliminated.

The second concerns the method of dispensing seawater into the enclosures. To increase the homogeneity of phytoplankton composition in enclosure seawater, it is strongly recommended that all enclosures are filled simultaneously with seawater at an identical flow rate. In our experiment, all nine enclosures were positioned in a row close to the mesocosm raft. Garden hoses connected each enclosure to a faucet installed near the bottom of a 2000 L seawater tank, which was filled with seawater pumped from approximately 4 m depth from a site adjacent to the raft and filtered through a 100 µm pore-sized filter before entering the tank. This procedure is likely to increase the probability that the initial composition of the phytoplankton community is similar across all enclosures (see Fig. 11). During the present study period, the bay water was vertically homogenous in terms of physical and chemical parameters, because of winter advective mixing.

The third factor concerns rapidly achieving the target pCO2 levels in seawater in the enclosures. To achieve pCO2 levels lower than the ambient concentration, we aerated seawater with pCO2-free air, while for pCO2 levels above ambient we aerated the seawater using air with pCO2 levels that were four-times higher than the target pCO2 concentration. This aeration protocol meant that seawater samples within all enclosures attained the target pCO2 levels within about 24 h (Fig. 7).

References


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