LIMNOLOGY
and
OCEANOGRAPHY: METHODS

Limnol. Oceanogr.: Methods 00, 2015, 00–00

doi: 10.1002/lom3.10055

A mesocosm concept for the simulation of near-natural shallow underwater climates: The Kiel Outdoor Benthocosms (KOB)

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Abstract

Biogenic, seasonal, and stochastic fluctuations at various scales characterize coastal marine habitats and modulate environmental stress. The relevance of most past studies into climate change impacts is weakened by the usually intentional exclusion of fluctuations from the experimental design. We describe a new outdoor mesocosm system for benthic research ("benthocosms") which permit the control and manipulation of several environmental variables while admitting all natural in situ fluctuations. This is achieved by continuously measuring the relevant variables (e.g., temperature, pH, O_2, CO_2) in situ, defining these in real time as reference values in the control software and simulating target climates by delta treatments. The latter constitute the manipulative addition of predefined changes (e.g., "warming", "acidification") to the reference values. We illustrate the performance of the system by presenting the environmental data of four seasonal experiments which together represent an entire year. The "Kiel Outdoor Benthocosms" allow realizing near-natural climate change experiments on complex benthic communities under controlled scenarios.

The impact of global environmental change on marine ecosystems has received exponential attention over the past decade. However, the great majority of manipulative experiments have been performed at the (sub-) organismal level, under constant lab conditions, and for short periods only (e.g., Wahl et al. 2015). It is increasingly recognized that while the insights obtained by these experiments have been important during the "juvenile" phase of climate change research, we now have to move forward by refining and upscaling our approach to more natural settings (e.g., Crain et al. 2008; Harley et al. 2012; Frieder et al. 2014; Waldbusser and Salisbury 2014). One particularly neglected but potentially important feature of the natural habitat are the fluctuations of the various environmental variables, including potential stressors (e.g., Shaw et al. 2012; Duarte et al. 2013; Gattuso et al. 2014; Wahl et al. 2015).

The extrapolation of the numerous lab findings from single ontogenetic stages to the entire life cycle of a species, from the organism to the community level, or from a constant to a stochastically or regularly fluctuating stress regime is unreliable and hinders our ability to make reasonable predictions. At the same time manipulative experiments in the field are most often financially and logistically unfeasible. As a compromise, Stewart et al. (2013) have strongly advocated the enhanced use of mesocosms for a more ecologically realistic climate change research. Large experimental units which approximate natural conditions offer the possibility to monitor and manipulate environmental factors and permit studying the response of interacting ecosystem components. Thus, mesocosms provide a powerful tool to close the gap between reductionist but analyzable laboratory experiments and natural but mainly observational field investigations. Extant mesocosm systems, however, do not generally allow controlled manipulation of the underwater climate

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Additional Supporting Information may be found in the online version of this article.

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including natural fluctuations as driven by season, hydrography and biology. Systems that come close to the requirement to add dynamic delta treatments to intrinsic natural fluctuations are the Free Ocean CO_2 Enrichment (FOCE) System (Kline et al. 2012; Gattuso et al. 2014; Kirkwood et al. 2015) and the mesocosms described by Jokiel et al. (2014a) as treated in more detail in the discussion.

Here we describe the "Kiel Outdoor Benthocosms (KOB)" facility which allows the application of "delta treatments" of several environmental factors. (The term "benthocosms" refers to the fact that this system is particularly suited for the work with benthic, rather than planktonic, communities.) The principle of this consists in shifting an environmental parameter (i.e., temperature, pH, oxygen, nutrients) to, for instance, levels predicted for the future while allowing it to fluctuate with natural frequency and amplitude. We deem it essential to allow these fluctuations since they have a recognized potential to modulate environmental stress (e.g., Benedetti-Cecchi 2003; Cornwall et al. 2013). Thus, fluctuations of a potentially stressful factor produce alternating phases of enhanced stress and relaxation of stress (Fig. 1). The former may increase mortality while the latter may facilitate survival. Intensity and duration of such positive or negative deviations from the mean will in the end determine whether fluctuations alleviate or aggravate stress relative to a condition without fluctuations. In addition, whenever the response to a factor is curvilinear, e.g., shows an intermediate optimum, according to Jensen's Inequality (Ruel and Ayres 1999) fluctuations in the vast majority of cases will lower an organism's performance. In any case, a fluctuating factor will affect most organisms differently than a constant factor with the same mean. Moreover, environmental fluctuations and stochastic processes have been shown to be particularly relevant for the dynamics of less abundant species (i.e., the ones at higher risk of – local – extinction; *see* Jordán et al. 2011). This notwithstanding, in most past experimental studies fluctuations were rather considered a nuisance and kept at a minimum in order to enhance the significance of an effect. In the very active research area of ocean acidification, for example, about 90% of the investigations applied the treatments in a constant mode (reviewed by Wahl et al. 2015) whereas in coastal habitats the daily as well as the seasonal fluctuations in pH may reach an amplitude of one pH unit (e.g., Saderne et al. 2013; Gattuso et al. 2014) and, thus, clearly exceed the expected anthropogenic shift of mean pH (ca 0.2–0.3 pH units) over the next 100 yr (Omstedt et al. 2012).

In view of the foregoing it seems crucial that in climate change research we adopt more realistic experimental concepts, i.e., simulate near-natural underwater scenarios with means and fluctuations corresponding to present and future settings. The delta treatments facilitated by the benthocosm infrastructure described below is one such possibility. In addition to the delta-treatments, the benthocosms allow for the necessary up-scaling from species to the community level under the influence of multiple, interactive stress parameters (e.g., warming, acidification, eutrophication, deoxygenation). When operated as a flow-through system, hydrographical background conditions including their natural fluctuations are carried into the experimental units and the manipulation of environmental parameters can be performed as delta treatments (e.g., "future" temperature = naturally fluctuating ambient temperature + x°C). In the following we describe the benthocosm hardware, its handling, seasonal CO_2 and temperature manipulations, the continuous monitoring and discrete sampling procedures, as well as additional features of the system. We further present a set of environmental data recorded during the first runs of the newly developed system.

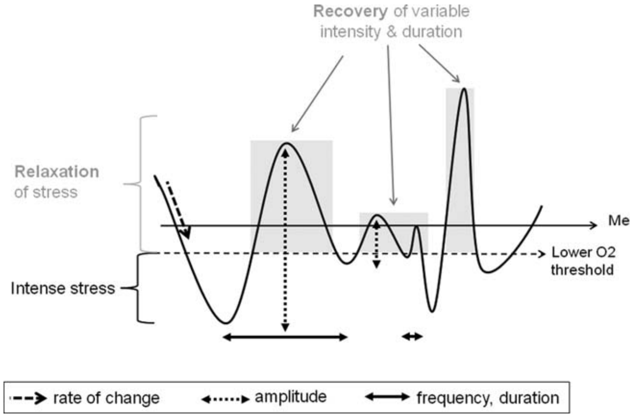

Fig. 1. An illustration of the attributes of fluctuations of an environmental parameter (potential stressor), here demonstrated with oxygen levels. Important attributes of fluctuations are the rate of change, its strength (amplitude), duration and the frequency (the number of changes of a certain size around the mean in a given time). An extreme event can be either very long or very strong or both and is usually defined beyond a certain threshold (e.g., above the 95 percentile of maximum temperature).

Material and procedures

General set-up

The KOB are located on an aluminum float (ALU-BAU, Büdelsdorf, Germany), moored to the peer of GEOMAR Helmholtz Centre for Ocean Research in the inner Kiel fjord (N 54°19.8′; E 010°09.0′). To accommodate variations in sea level, the mooring consist of wheel-sledges sliding along vertically mounted steel rails fixed to the pilings of the pier. An overview of the benthocosm facility and the main components of a single tank are given in Fig. 2a,b. The six benthocosms are made of recyclable and UV-resistant polyethylene (PE) tanks with a volume of 3000 L (Heers & Brockstedt Umwelttechnik, D-24539 Büdelsdorf). The benthocosm tanks are built of double walled PE (inner dimensions: 2 m × 2 m × 0.9 m) with a 5 cm thick styrofoam layer between inner

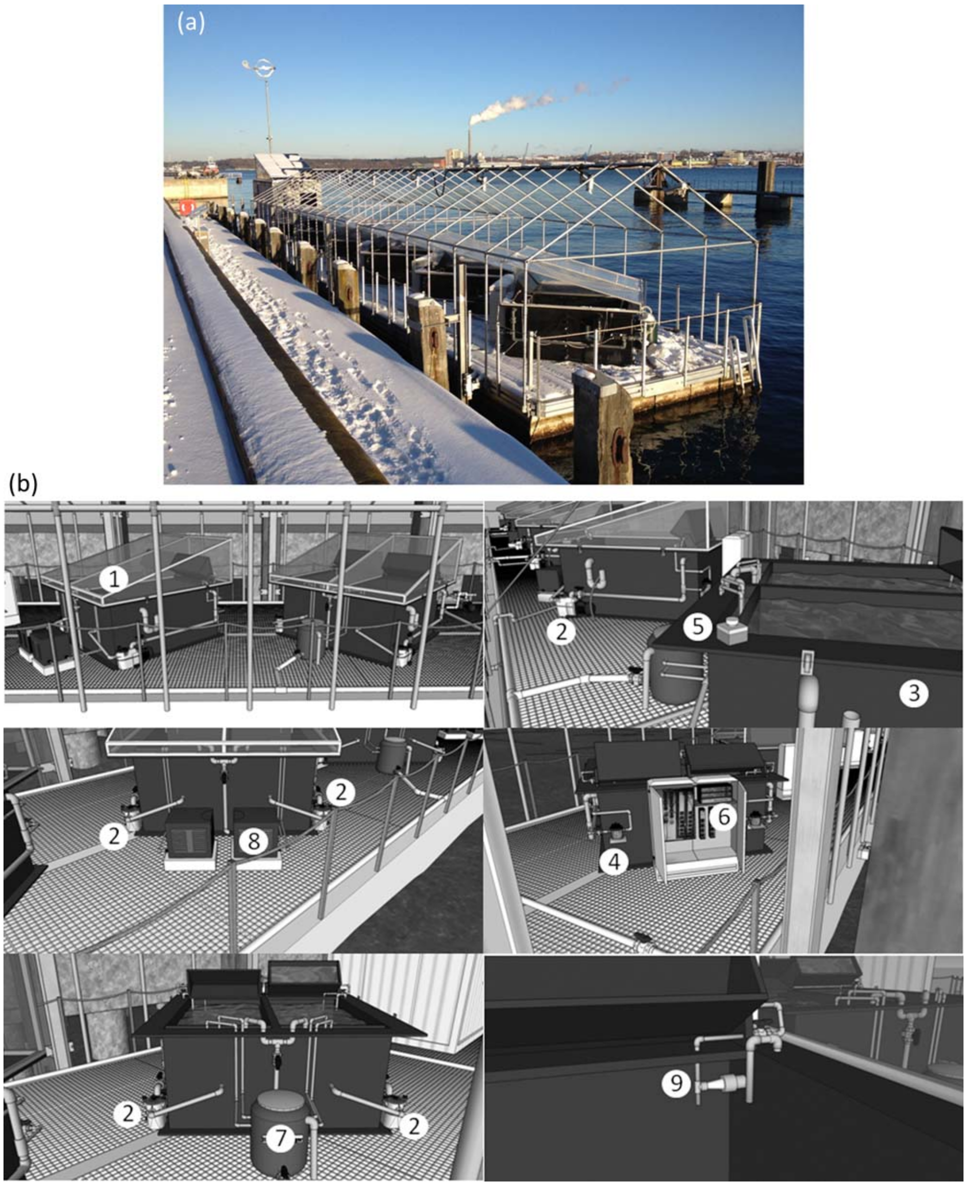

Fig. 2. (a) Overview of the Kiel Outdoor Benthocosm infrastructure showing the six tanks with their subunits covered by hoods as well as (in the background) the technical container bearing on its roof the solar panels and the wind generator. (b) Sketches of important functional components of KOB. Not shown are the numerous IT components. 1: hood, 2: circulatory pumps, 3: position of lateral ports, 4: measurement cell, 5: pCO_2 sensor, 6: measurement and control unit, 7: heat exchanger, 8: coolers, 9: water inflow (and air injection (not shown)).

and outer wall to ensure best thermal insulation. Six tanks, each of which can be divided into two subunits (volume: 1500 L) by means of a double-walled transparent PVC separation, allow for running experiments with up to 12 replicates (depending on the number of factors and treatment levels). These 12 independently controlled units can either be operated in a "regression mode" (e.g., 6–12 climate change scenarios representing different target years or a gradient of increasingly severe assumptions, no replication) or in a factorial "ANOVA mode" with present vs. future climate scenarios (3–6 replicates). The two neighboring subunits are independent of each other in every aspect except with regard to temperature. Due to the limited insulating capacity of the separating mid-wall temperature differences of only up to 5°C can be permanently maintained between the two subunits of a unit. The benthocosms can be operated in closed-circuit mode or an open-circuit mode (flow-through of natural seawater), with water pumped from Kiel Fjord at 1 m water depth (Universal pump 1262 910, EHEIM, Deizisau, Germany). Additional features (described in more detail below) of the Benthocosms are a translucent hood (#1 in Fig. 2b) to enclose a headspace with controllable gas

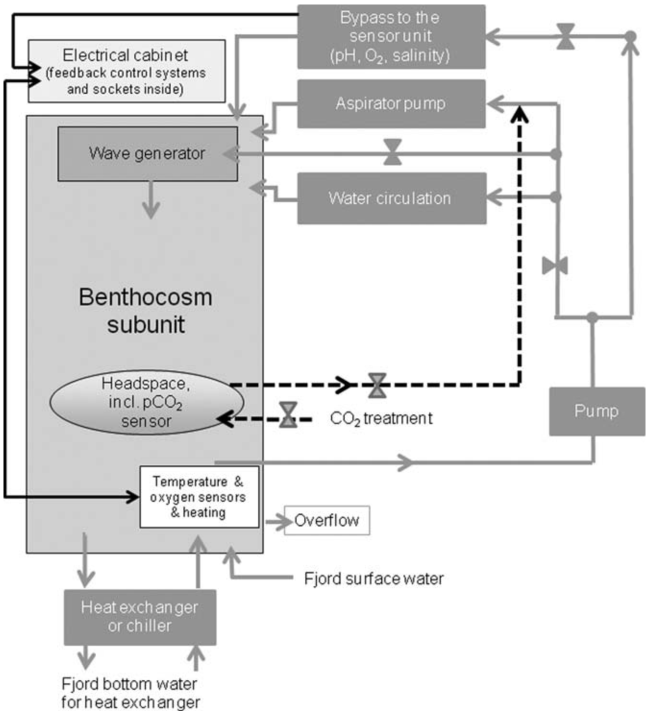

Fig. 3. Schematic showing the control elements of a single tank subunit.

composition, an automated heating and cooling system, continuous water supply from Kiel fjord, a wave generator, a remote video control unit (Panasonic WV-SP105 and Cam IQ Satellite Manager from Rosemann Software) and diverse sensors to monitor environmental parameters. A sketch of the control systems is given in Fig. 3.

A circulation pump (ATK-MP10041 or Kripsol OK33B, 10^4 L h^{-1}, (#2 in Fig. 2b) is used to produce currents inside the benthocosms to further mimic water movements as close as possible to natural conditions, and to distribute heat or injected nutrients in the water body. The mixing efficiency was tested with 50ml color tracer (calcein: 1.5 g L^{-1} artificial sea water). Without circulation pump and wave generator the water column and the tracer were homogeneously mixed within 10 min (as assessed by eye), with circulation pump and without wave generator the requested time for homogeneous mixing was 3 min, with circulation pump and wave generator mixing took 2 min (Supporting Information Fig. 2). Additionally, the water dilution in the benthocosms due to the preset flow-through (about one tank volume per 24 h) was tested by adding a concentrated nutrient solution through one of the side ports (#3 in Fig. 2c) at 9:00 and measuring the decrease in nutrient concentrations in the tank every other hour. In the absence of any macrobiota, half of the injected nutrients were washed out within 4–6 h (Fig. 4).

A reactor chamber (#4 in Fig. 2b) for the injection of gases into the circulating benthocosm water under counter current conditions, allows for various gas treatments (e.g., oxygenation or deoxygenation).

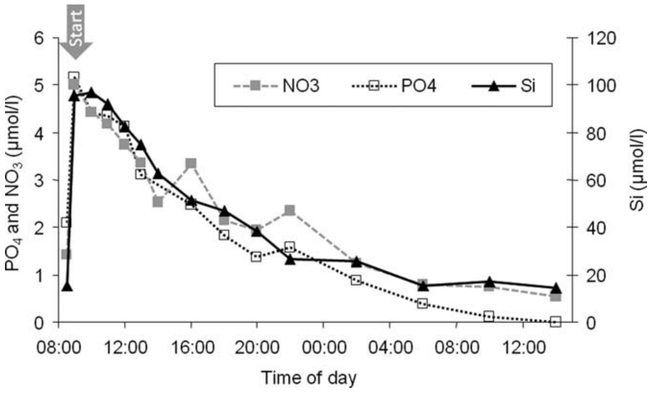

Fig. 4. Decrease of injected ("start") nutrients caused by a flow-through of fjord water at 1500 l/24 h in a tank devoid of biota. The earliest values are the nutrient concentrations in the tanks before injection.

With removed hoods, a remote video observation system allows for an on-line control of the benthocosms mechanical functioning and enables the observation of water motion after the injection of dyes (used to assess mixing dynamics). The benthocosm facilities partially (~30%) draw their energy from autonomous, renewable sources (solar panel: Sun Nord Neue Energien GmbH, Kiel; wind turbine: Firma AIE—Alternative und Innovative Energieberatungen GmbH, Cuxhaven, both Germany).

The target organisms or communities inside the benthocosms are placed on a grating that is suspended in the tanks at optional water depths between −10 cm and −70 cm. During single experimental runs (10–11 weeks) uncontrolled recruitment onto the inner walls of the tanks was permitted but usually controlled to some degree by the grazers of the community.

Carbonate chemistry manipulation

Each unit is covered with a hood made of a gas-tight, transparent foil (Lumisol clear, Folitec, Westerburg, Germany). The foil, supported by an aluminum frame, isolates a headspace above each subunit (volume: 1632 L) from the surrounding atmosphere. Within the headspace the partial pressure of carbon dioxide (pCO_2) can be manipulated to simulate increasing atmospheric pCO_2 levels. This CO_2 manipulation causes an acidification signal in the benthocosm water by air-sea gas exchange. The CO_2 exchange between the headspace and the benthocosm water is enhanced by waves that are generated by the periodical emptying of a continuously refilled water chamber (frequency: 30 waves h^{-1}; volume: 23 L $wave^{-1}$) and additionally, by the injection of air from the headspace into the internal water cycle by means of an aspirator pump (Venturi effect). The approach of creating ocean acidification by maintaining elevated pCO_2 levels in the headspace instead of adding acid or

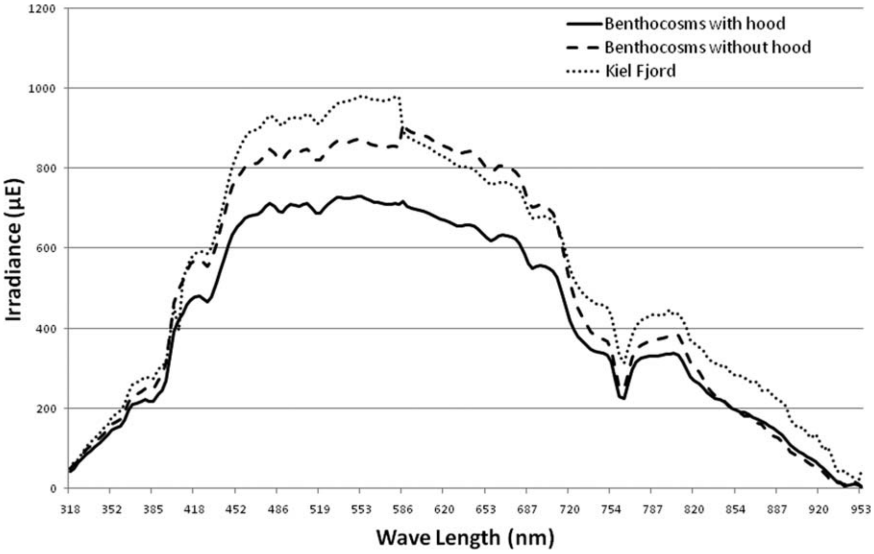

Fig. 5. Solar irradiance (13 August 2012, noon) measured at −0.5 m water depth in the fjord, and within the tanks with and without hood.

bubbling the tank water itself with CO_2-enriched air has three important advantages: (1) it simulates the real atmosphere-ocean exchange, (2) the treatment does not suppress the biogenic fluctuations of the carbonate system driven by the communities in the tanks (see Assessment part) and (3) it does not change the alkalinity. The pCO_2 inside the headspace is automatically controlled by injecting CO_2 until a predefined treatment level is reached in the hood atmosphere (Control unit: Scenty, HTK, IR Spectroscopy, Hamburg, Germany; CO_2: Lindegas, Pullach, Germany, (#5 in Fig. 2b)). Whenever pCO2 falls below a lower threshold, more CO2 is injected. Lower and upper thresholds are freely selectable. In the present set of experiments we chose the thresholds such that the head space of the high-pCO_2 tanks had a mean value of 1100 ppm. The overall mean effect of head space enrichment with CO_2 from ambient (380–450 μatm) to 1050–1100 μatm was a pH reduction of the tank water by 0.18 (SD 0.08. CI 0.04) pH units (*see* Supporting Information Fig. 3). The hoods over those tanks that were to be run at today's atmospheric conditions had openings in their side walls to permit free circulation of air.

The hood's roof is inclined at a 15° angle towards the south to maximize the penetration of sunlight, to reduce condensation on the inner side and the trapping of rain water or snow on the outside. The foil of the hood permits all wavelengths of the solar spectrum between 300 nm and 1000 nm to penetrate at similar proportion (Fig. 5) as measured inside the tanks at 0.5 m water depth by a UV-VIS Radiance Sensor (Ramses-ACC-VIS, 320-950 nm, TriOS Science, Germany). Across all wave lengths the average adsorption by the foil material is 7%, and in the photosynthetically relevant spectral regions (430–440, 480–550, 650–670 nm) it is, on average, 17%. This decrease approximates the attenuation by a 0.5 m thick layer of Kiel fjord water (Wahl unpubl. data).

Temperature manipulation

Water temperature is controlled by an aquarium controller (Profilux-3ex and Expansion Box, powerbars of the types 6D PAB & STDL4-4. GHL Advanced Technology, Kaiserslautern, Germany, #6 in Fig. 2b). The powerbars represent a total of 10 controllable power sockets per benthocosm subunit. The expansion box offers the possibility to connect further sensors for the control of additional factors. Twelve of these aquarium controllers serve the benthocosm subunits, and an additional one measures temperature and pH (or salinity and oxygen if required) of the Kiel Fjord water. All controllers are connected serially using the Profilux Aquatic Bus (PAB). The PAB enables communication between the individual units, allowing the very important and unique feature of the dynamic nominal value. The dynamic nominal value component within the software (GHL Profilux Control or GHL Control Center) was specifically designed for the KOB, and has become a standard feature by now. To date, two different modes can be run. In the dynamic delta treatment mode, the reference nominal value is a measured value from a sensor in the reference system (measured in Kiel Fjord in this case) transmitted to the other controllers which then use it as a reference value for the delta treatment, i.e., relative to which a tank is warmed or cooled to a preset delta. This allows "treating" the system while admitting the stochastic or seasonal fluctuations of the fjord water. In the fixed programme mode, up to 1000 target temperature values can be entered for each individual tank with temporal resolution from seconds to weeks between subsequent target values. The temperature slopes between two subsequent target values are linearly interpolated by the software. The system possesses an alarm notification via e-mail (default) or with an upgrade via SMS. The Profilux-3ex offers the possibility to log sensor data as text file. Temperature and pH values in our case were logged every 10 min, but this interval can be chosen at will.

To avoid deviations from the target temperature by solar irradiation or by large temperature differences between water and air, the KOB units are equipped for cooling and heating at all temperature treatment levels. Cooling of the benthocosms is accomplished by heat exchange (#7 in Fig. 2b) between the internally circulating benthocosm water (Universal pump 1262 910, EHEIM, Deizisau, Germany) and colder bottom waters pumped at controlled rates from the fjord at 15 m water depth (Badu 90/20 AK-Dr, Speck pumps, Neunkirchen am Sand, Germany). During seasons, when the temperature difference between bottom and surface water in the fjord is too small for cooling the tanks, cooling is achieved by electrical heat exchangers (Titan 2000, Aqua Medic, Bissendorf, Germany, #8 in Fig. 2b). For heating, each benthocosm subunit is equipped with three internal heating elements (600 W, Schego Titan, Schemel & Goetz, Offenbach am Main, Germany). Heaters, coolers and the pumping of cold bottom waters are automatically regulated by the above-mentioned aquarium controller. In this manner, seasonal, daily or convection-caused temperature fluctuations at natural frequencies and amplitudes are admitted to the tanks whether they run at today's or tomorrow's temperature regime.

Measurement of environmental parameters

Temperature and pH in the benthocosm are continuously logged by sensors (platinum resistance thermometer PT1000 and gel-electrolyte filled glass electrode, resp., both from GHL Advanced Technology, Kaiserslautern, Germany). pCO_2 is monitored in the head space under the hood (see above). Oxygen concentration in the water phase is logged continuously using dissolved oxygen optodes (LDO Hach-Lange, 4H.Jena engineering GmbH, Jena, Germany). Salinity is continuously logged in the fjord by GEOMAR. Discrete salinity measurements in the KBOs with portable conductivity meters (WTW Cond 3110 + Tetra Con 325, Wissenschaftlich Technische Werkstätten, Weilheim, Germany) showed that there was no significant difference between the salinity in the fjord and in the benthocosms (due to continuous flow-through). All environmental parameters are logged in the tanks (or the head space of the tanks) as well as in situ in the Kiel Fjord from which the flow-through supply is pumped. This means that at each preset time interval 4–5 environmental variables are measured by as many sensors at 13 points (12 experimental subunits plus fjord), fed to the central computer and regularly and automatically transferred via email to a central server for data visualization and storage. Data are extracted from the emails and processed by a script using the R programming language (version 3.1.0, cran.r-project.org). The data are tabulated and stored in text files. The R script automatically generates line diagrams from these data, showing the histories of the measured parameters for each subunit with 2-h resolution. The tabulated data and the diagrams are automatically uploaded to a Dropbox account (www.dropbox.com) for real-time online access by the project participants.

To check for sensor drift pH, and O_2 are measured daily using hand-held and calibrated sensors of the models Seven Multi + InLab Expert Pro (pH, Mettler Toledo GmbH, Giessen, Germany), and Multi WTW Oxi 3515 + FDO 925 (oxygen, Wissenschaftlich Technische Werkstätten, Weilheim, Germany). The pH electrode was calibrated with NBS pH-buffer (4.001, 6.865). Indeed, the pH loggers tend to deteriorate over time and must be replaced every 3–6 months. During this aging process their accuracy drifts. We correct for this drift in the following manner. First, we regress the daily measurements of the reference sensor from Mettler Toledo (MT) (y) and the value logged at the same point in time by GHL (x). When the GHL values explain more than 65% of the MT values, we use the regression model directly to correct the GHL values. When the R^2 is below 0.65, we calculate the difference in proton concentration (= power (10, -pH)) between the drifting pH sensor from GHL and the calibrated pH sensor from MT for each day of the experiments. Subsequently the relationship between this difference and time is modeled using the "fitting functions" procedure in Statistica (Statistica 8.0, StatSoft, Tulsa). This function (typically a quadratic polynomial) is used to correct the GHL values. Then we regress once more the corrected GHL values (x) by the reference values (Mettler Toledo) (y). In the vast majority of cases, the corrected GHL values explain more than 80% of the daily variance of the MT values. When after this correction, the regression coefficient is still < 0.65, the GHL sensor is considered broken and replaced at the earliest convenience.

In situ water pCO_2, pH, temperature and O_2 are measured continuously by carbon dioxide sensor (Hydro C CO_2, Contros, Kiel, Germany) combined to a SeapHOx unit (pH-O_2-salinity sensor package, Scripps Institution, San Diego) attached to the side of the floating platform at 1m depth in the fjord. In May and June 2014, a transiently available second Hydro C CO_2 unit permitted assessing pCO_2 sequentially in differently treated tanks and continuously and synchronously in the fjord.

Besides these time-series data, regular discrete water sampling and subsequent analysis were performed for total alkalinity (TA) and nutrients (twice per week) as well as for dissolved inorganic carbon (DIC, monthly). The water samples for these measurements were drawn from sampling ports that are positioned in a vertical row every 10 cm depth of the tank walls (#3 in Fig. 2b). Water samples for DIC analysis were filled bubble-free into 50 mL Winkler bottles, poisoned by the addition of one drop of saturated mercury chloride ($HgCl_2$) solution and measured via coulometric titration (Johnson et al. 1993; Winde et al. 2014). TA samples were filtered through a 0.45 μm Minisart syringe filter (Sartorius SFCA—Surfactant-Free Cellulose Acetate, Sartorius AG, Göttingen, Germany) and measured using a titration unit with associated sample changer (Titroline alpha plus, SI Analytical Mainz, Germany; Winde et al. 2014). DIC and TA measurements were calibrated using certified seawater standards (Dickson, Scripps Institution of Oceanography, San Diego; Dickson et al. 2003, 2007). The precision in the runs presented hereafter was better than ± 2 μmol/kg for DIC and better than ± 10 μmol/kg for TA. Nutrient samples were filtered through 0.45 μm Minisart syringe filter (Sartorius SFCA, Sartorius, Göttingen, Germany) and stored frozen (-20°C) until measured using a QuAAtro nutrient analyzer (SEAL Analytical GmbH, Norderstedt, Germany; Winde et al. 2014) were performed. Accuracy and precision were verified by replicate analyses of a solution from powdered ammonium-, phosphate-, and nitrate salts. The precision in the runs presented hereafter were better than 8% RSD (relative standard deviation). The high quality of pH calculations is illustrated in Supporting Information Fig. 3.

Assessment of performance

In 2013 and 2014 we ran four subsequent seasonal experiments of 9–12 weeks duration each in order to assess the effects of acidification and warming under near natural conditions at organismal to community levels. The successive experiments ran in the following periods: spring 04 April 2013–19 June 2013, summer 04 July 2013–17 September 2013, autumn 10 October 2013–17 December 2013, winter 16 January 2014–01 April 2014. Here we only report on the behaviour of the abiotic components as driven by (1) the fluctuations in the fjord system, (2) the controlled delta treatments and (3) the biological activity (mainly photosynthesis and respiration) of the biota in the tanks. As a rough indication of the biotic drivers in the tank it should be mentioned here that each subunit contained 20–25 individuals of the macroalga *Fucus vesiculosus* (ca 2600 g WW), 25 individuals of the blue mussel *Mytilus edulis* (total of 320 g WW), 2 individuals of the seastar *Asterias rubens* (total of 30 g WW), ca 60 individuals of the snail *Littorina littorea*, ca 43 individuals of the isopod genus *Idotea* spp., and ca 100 individuals of the amphipod genus *Gammarus* spp. and a non-quantified amount of species recruiting during the experimental runs (filamentous algae, juvenile barnacles and mussel, etc.). The biomass added varied slightly between experimental runs but was always equal between all subunits of a given run. When assembling the communities we tried to mimic the specific abundances as encountered at the sampling site. Between the four seasonal runs, the tanks were emptied and cleaned.

In all seasonal runs, the units were subjected to crossed warming (+5°C relative to fjord water) and acidification (1100 μatm pCO_2) treatments. The warming of the Baltic Sea until the end of the century is expected in a range of 3–6°C (Helcom 2007; Graewe et al. 2013) which is corroborated by an observed multidecadal warming trend in this region of 0.5–1°C per decade since the second half of the 20th century (Elken et al. 2015). Consequently, an assumed warming by 5°C over the next 100 yr may sound severe but is realistic for the shallow coastal Baltic habitats. Three tanks ran at ambient fjord water temperature, the remaining three tanks at temperatures corresponding to the expected conditions in 2115. Within each tank, one of the two subunits was acidified by maintaining the headspace atmosphere at 1100 μatm CO_2, the other subunit headspace was open to the outside atmosphere (380–400 μatm CO_2). The flow-through of fjord water corresponded to 1.0–1.3 times the tank volume (1500 L) per 24 h.

Several 24-h sampling campaigns were conducted to follow short-time variations in the benthocosm (and the fjord) under the impact of seasonally changing conditions of light, temperature and biological activity. In these high-resolution campaigns, every other hour differently treated subunits were sampled. pH, salinity, temperature and oxygen were measured and aliquots were taken for lab-based measurements of the carbonate system, nutrients, and metals. The systems ran in normal experimental set-up modus with closed hood, and the sampling was conducted through a tube with a two-way valve at a port in the tank wall.

Stable isotope signatures, major and trace elements concentrations, both in the water column, as well as all the physiological, biological and ecological responses to the treatments will be reported elsewhere. In the following we describe the abiotic dynamics in the benthocosms to illustrate their functioning.

Temperature—pCO_2 factorial experiment over 1 yr

Temperature showed a seasonal variation typical for the region with a minimum of about 1°C in early February and a maximum of about 24°C in late July (Fig. 6) with the maximum temperatures in summer represent an unusually pronounced heat wave (Logged raw data at http://doi.pangaea.de/10.1594/PANGAEA.842739). In addition, daily fluctuations in the order of 1–3°C were observed, reaching extremes of 5°C on sunny summer days. The temperature in the tanks of the "ambient" treatment followed the in situ temperature in the fjord very closely. The "delta-warming" treatment led to an increase of mean temperature by approximately 5°C but did not affect frequency or amplitude of the seasonal or daily fluctuations. This led, for instance, to a minimum temperature of 6°C at the end of January and a maximum of 29°C at the end of July (during the heat wave) in the warmed treatments.

In situ (Kiel Fjord) pH varied seasonally between 8.5 in spring (April–June) and 7.7 in autumn (October–November) (Fig. 8a,b). Note that the in situ values recorded before 30th March 2013, as depicted in Fig. 7, were measured at a

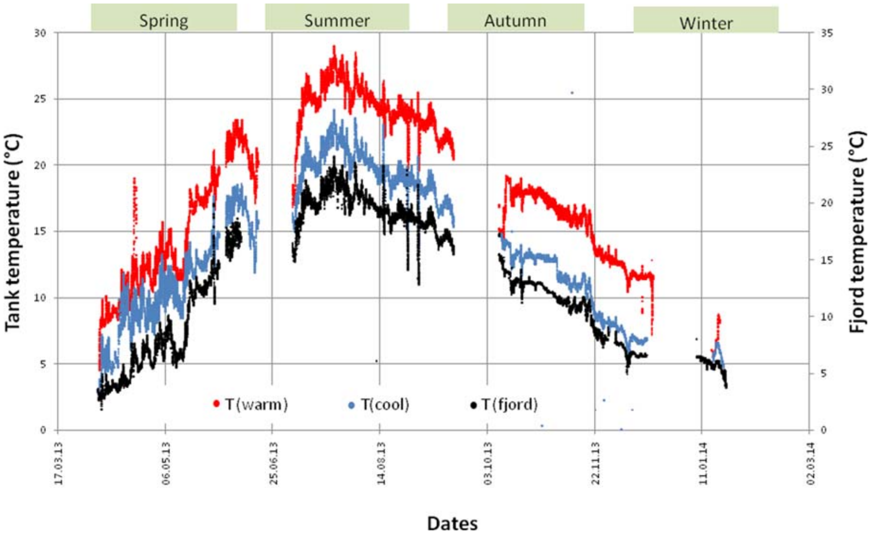

Fig. 6. Temperature in a warm tank (red), a cool tank (blue) and the fjord (black, scale set off deliberately to avoid the identical cool tank and fjord temperatures masking each other).

shallower depth (−0.15 m) than the inlet of the flow-through pump (−1 m). After that date in situ values were taken in the immediate neighborhood of the inlet. In situ pCO_2 fluctuated daily by 100–300 μatm. In the tanks, with their abundant algal biomass, the circadian amplitude (despite continuous flow-through of fjord water) was 2–5 times larger (Fig. 7). These biogenic fluctuations varied in amplitude among treatment combination and season (Supporting Information Fig. 4) and ranged between 0.15 and 0.5 pH units with an overall mean amplitude of 0.32 (SD 0.12). The range of these diurnal amplitudes of pH fluctuation correspond well with those observed in natural *Fucus* stands (Middelboe and Hansen 2007; Saderne and Wahl 2013; Saderne et al. 2013 and own unpublished data shown in Supporting Information Fig. 5: 0.6–1.2 pH units) as well as in kelp forests (Frieder et al. 2012), coralline boundary layers (Hurd and Pilditch 2011; Cornwall et al. 2013), rock pools (Morris and Taylor 1983), and seagrass habitats (Semesi et al. 2009). pH reached a maximum during late afternoon and a minimum in early morning, i.e., at the end of photosynthesis and respiration dominated phases, respectively (Supporting Information Fig. 5). The difference between these extremes in the season (spring) was 0.2–0.4 pH units (Supporting Information Fig. 4). The increase of the headspace CO_2 concentrations above the water body at ambient temperature lowered the water pH by, on average, 0.2 units (Supporting Information Fig. 6) which corresponds well to the expected acidification trends for the coming century (Omstedt et al. 2012). This effect was most pronounced in the winter/spring experiments. It should be noted, that the seasonal and daily pH variations in the CO_2 enriched and non-enriched systems were of similar amplitude, but pH at both acidification levels was lower under warmer temperatures (Fig. 7, Supporting Information Fig. 4). pCO_2 in the fjord water varied between 300 μatm and 1100 μatm in May and June 2014 with a mean day-night amplitude of about 200 μatm presumably driven by photosynthesis-respiration cycles in the plankton (Supporting Information Fig. 7). The pCO_2 in the tank water was controlled by the treatments and the day night amplitudes are influenced by the biomass in the tanks. Warming seemed to increase the day-night amplitudes of pCO_2 stronger than acidification did. Their combined effect produced the largest amplitudes, but these were smaller than the sum of single effects and must, thus, be considered antagonistic.

Despite the flow-through rate of approximately one tank volume exchange per 24 h, pH, pCO_2, TA and DIC differed substantially between the tanks (treatments) and the fjord illustrating the strong influence of enhanced biological activity in the tanks (Figs. 8, 9). TA and—more pronounced—DIC were lowest in late summer (August) and highest in winter (January–March) in situ as well as in the benthocosms (Fig. 9). During spring bloom and late summer bloom the primary production was so high that the water was CO_2 undersaturated (100–350 μatm), and represented a possible sink for CO_2. During autumn and winter (September–March) the water column was CO_2 supersaturated (500–1000 μatm) and thus constitutes a potential source for atmospheric CO_2 for the atmosphere. Phytoplankton blooms are responsible for the pH maxima in the seasonal trend, superimposed by varying impact by North Sea water inflows and discharge-dependent mixing with fresh water from the a small, nearby river, the Schwentine. The mixing processes with both North Sea water and Schwentine river water were responsible for the DIC and TA maxima during winter time both in the Kiel

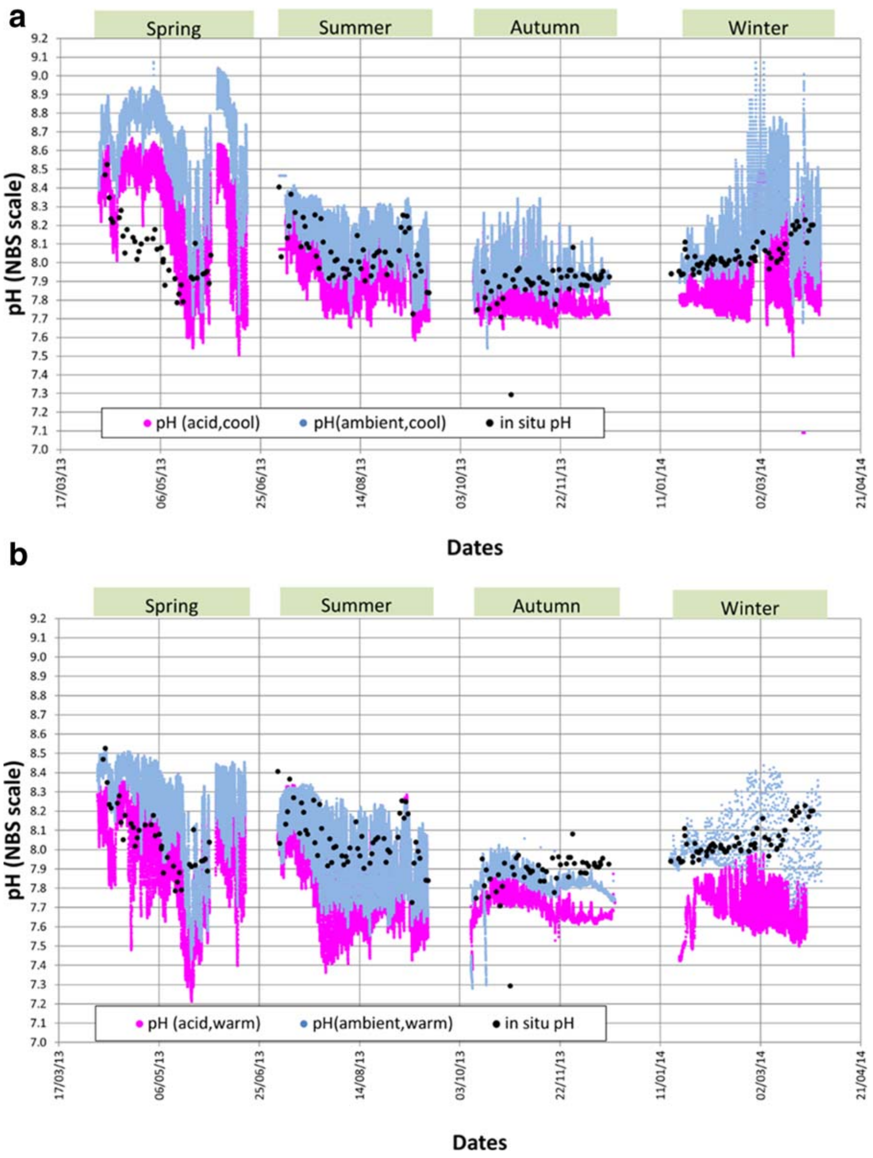

Fig. 7. (a) pH in the acidified (pink) and non-acidified (blue) subunits of a cool tank and in the fjord (in situ, black). (b) pH in the acidified (pink) and non-acidified (blue) subunits of a warm tank and in the fjord (in situ, black). Note that the Fjord pH measurements prior to 30th May were taken at a shallower depth than the inlet of the flow-through. After that date the pH in the Fjord was measured close to the inlet.

Bight (e.g., Winde et al. 2014). Additionally, upwelling of deeper nutrient-rich waters may have caused changes in the nutrient and carbonate systems, and the pH of the Kiel fjord surface waters (Feisel et al. 2008; Nikulina et al. 2008). In the benthocosms containing the macroalgae-based communities, the in situ seasonal (and stochastic) fluctuations were modified by the biological activity typically leading to a removal of DIC from the solution and a rise in mean pH as illustrated in the 24 h profile (Fig. 9).

Nutrient concentrations in the mesocosms, exemplified by the concentrations of PO_4 and NO_3, followed the seasonal fluctuations in the fjord but were reduced by the uptake by primary producers (macro- and microalgae) (Fig. 9). It should be kept in mind that transport processes during biologically active phases may produce microscale changes in pH and the carbonate system close to the thallus surface of the Fucus plants that may differ from the values of the aqueous bulk solution (e.g., Cornwall et al. 2013; Wahl et al. 2015).

Discussion

Of the recent 267 articles describing mesocosm work as identified by Stewart et al. (2013) only four systems seem suitable for replicated studies on marine benthos in medium to large units (>100 L) for medium to long duration (>1

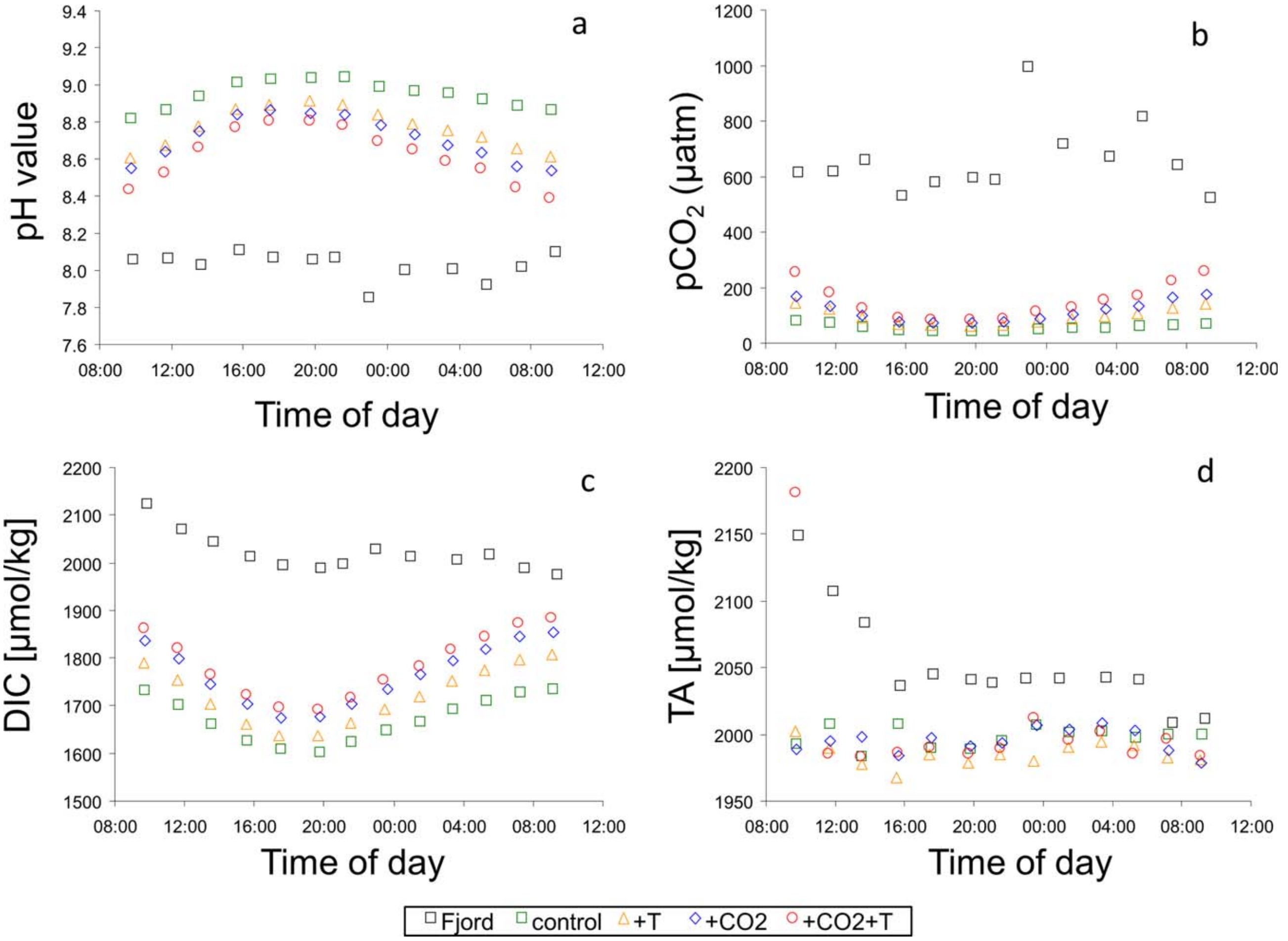

Fig. 8. Evolution of pH (a), pCO_2 (b), DIC (c) and TA (d) during a 24 h photosynthesis-respiration cycle under in situ conditions and the various treatment combinations as assessed on on 24th and 25th of April 2013.

month). Two additional systems were identified in Norway and Estonia. The properties of these systems are presented along with the KOB in Supporting Information Table 1. It becomes obvious, that in comparison to these existing systems, the KOB represent a true innovation regarding their combined qualities in experimental unit volume and number, admittance of natural conditions, controlled multifactorial delta treatments and real time logging of relevant variables. Thus, the KOB benthocosm system allows for experiments under near-natural conditions on numerous aspects of stress ecology in general and climate change in particular. Most importantly, they permit the combination of natural fluctuations with controlled (delta-) treatments.

Two more recently developed mesocosm systems allow controlled manipulation of the underwater climate including natural fluctuations as driven by season, hydrography and biological processes. The in situ CO_2 enrichment system FOCE, that maintains the experimental pH as an offset from the environmental pH (Gattuso et al. 2014) was deployed in coral reefs, seagrass meadows, sand flats, polar regions and in deep sea (Kline et al. 2012; Barry et al. 2014; Gattuso et al. 2014; Kirkwood et al. 2015). While FOCE enables community level experiments for relatively long periods of time, it is limited to one single factor (pH) and lacks an adequate replication, thus may not provide statistically sound results. Furthermore, FOCE systems incur high cost and therefore its usage is not probable to most researchers. Another aquatic mesocosm featuring environmental fluctuations was recently described by Jokiel et al. (2014a). Similarly to KOB facility, this is a large-volume flow-through mesocosm system, manipulating both temperature and pH while allowing a constant offset from the diurnal and seasonal temperature regime. Operating at low cost, this system permits long-term experiments using replicated design (Jokiel et al. 2014b). The key difference between KOB and the system described by Jokiel et al. (2014a) is the CO_2 enhancement mechanism—atmospheric pCO_2 manipulation vs. seawater CO_2 bubbling. In addition, the Jokiel system seems not equipped to dynamically manipulate O_2 and to program

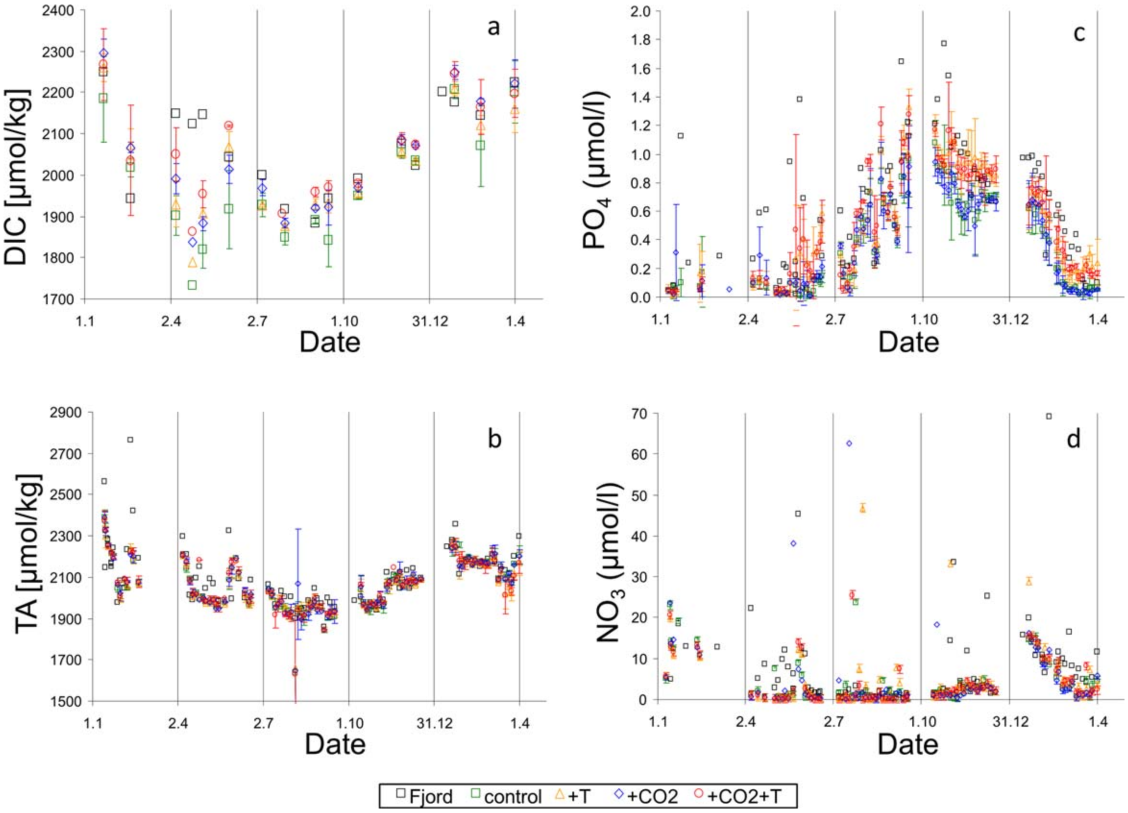

Fig. 9. DIC (panel a), TA (panel b) phosphate © and nitrate (D) in the fjord (in situ) and in the differently treated tanks (error bars represent SD).

natural and/or manipulated temperature regimes of entire years.

The fluctuations in temperature, salinity, nutrients, and various components of the carbonate system as driven by season, hydrography (including up- and down-welling) and biology (photosynthesis, respiration and calcification, mainly) have manifold consequences for aquatic biogeochemistry, marine communities and their components. Manipulations of separate variables (e.g., gaseous pCO_2, temperature, nutrient concentrations) may cause oscillations between beneficial and stressful values. Parallel fluctuations of multiple variables either synchronously or asynchronously, may led to the compensation of stressful levels of one variable that may hinder an organism in, for instance, using resources which are at beneficial levels or may enhance sensitivity to other stresses, or —vice versa— plentiful resources may facilitate the tolerance of stressful conditions (e.g., Thomsen et al. 2010; Melzner et al. 2013; Frieder et al. 2014). Fluctuations may generally decrease an organism's overall performance (Ruel and Ayres 1999; Benedetti-Cecchi 2003) but also offer temporal refuges from stress (Wahl et al. in press). Finally, since species respond differently to given levels of resources or stress, environmental fluctuations may lead to oscillating competitive hierarchies, thus facilitating coexistence and diversity (sensu Namba 1984). Given this ecological importance of environmental fluctuations and their ubiquitous existence in coastal environments (e.g., Duarte et al. 2013; Waldbusser and Salisbury 2014), relevant investigations on e.g., climate change should allow, instead of exclude, fluctuations in the experiments. At the same time it is desirable that factors of interest (temperature, nutrients, pH, salinity.) could be manipulated and controlled while maintaining frequency and amplitude of their fluctuations. The benthocosm system presented here meets these requirements quite well. The total costs for the KOB in the state described here were approximately 600 K Euro in 2015.

Outlook

At the moment dynamic delta treatments of temperature and pCO_2 work fulfill all requirements. Similarly the recently realized fine-resolution programming of entire temperature regimes open new possibilities of exploring extreme events. Some features will be implemented in the near future: delta-treatments of oxygen by a combination of reliable O_2-logging and programmable bubbling, the simulation of upwelling events by alternating the flow-through between surface and deep water, which, in late summer, usually are hypoxic, and the manipulation of the nutrient regime, the latter may be the most challenging project since several questions regarding automated monitoring and control of single or combined nutrient levels are still unresolved.

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Acknowledgments

We acknowledge N. Wendländer for technical support during sampling and Leibniz IOW for further support. The present study was carried out in the frame of the collaborative BMBF projects "BIOACID 2" and "BaltMed." We highly appreciate the most conscientious participation in this project of the students Balsam Al-Janabi, Angelika Graiff, Birte Mensch, Andreas Pansch, Finn Petersen, Stephanie Raddatz and Franziska Werner. Without their enthusiasm and omnipresent willingness to watch over the system even on weekends, this complex infrastructure would certainly have run less smoothly. An earlier version of this article was substantially improved by the valuable comments of two anonymous reviewers and the associate editor George Waldbusser. We greatly appreciated their input.

Submitted 17 February 2015
Revised 7 June 2015
Accepted 19 June 2015

Associate editor: George Waldbusser