



In situ incubations with the Gothenburg benthic chamber landers: Applications and quality control

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ABSTRACT

In situ incubations of sediment with overlying water provide valuable and consistent information about benthic fluxes and processes at the sediment-water interface. In this paper, we describe our experiences and a variety of applications from the last 14 years and 308 deployments with the Gothenburg benthic chamber lander systems. We give examples of how we use sensor measurements for chamber leakage control, in situ chamber volume determination, control of syringe sampling times, sediment resuspension and stirring quality. We present examples of incubation data for in situ measurements of benthic fluxes of oxygen, dissolved inorganic carbon, nutrients, metals and gases made with our chamber landers, as well as manipulative injection experiments to study nitrogen cycling (injections of ¹⁵N nitrate), phosphate retention (injections of marl suspension) and targeted sediment resuspension. Our main goal is to demonstrate the possibilities that benthic chamber lander systems offer to measure solute fluxes and study processes at the sediment-water interface. Based on our experience, we recommend procedures to be used in order to obtain high quality data with benthic chamber landers.

1. Introduction

Biological, chemical, physical and geological processes that take place at or near the seafloor influence and regulate the functioning of aquatic environments and determine the fate of biogenic material deposited from the photic zone (e.g. Aller, 2014; Rowe et al., 1975). Soft sediments constitute a major sink for many elements essential for life (such as C, N, P, Si and trace elements) and host an enormous biodiversity of bottom dwelling animals (Snelgrove, 1999). Pelagic ecosystems are often heavily influenced by the exchange of substances across the sediment-water interface (Griffiths et al., 2017). For example, bottom sediments may act as substantial sources of regenerated nutrients, fuelling primary productivity (Bonaglia et al., 2017; Ekeröth et al., 2016a), or release pollutants to the water column (e.g. Covelli et al.,

1999; Schaanning et al., 2006). The quantification of solute exchange rates between the sediment and the overlying water, herein ‘benthic fluxes’, is therefore a crucial task for marine scientists. Another value of benthic fluxes is that they provide a robust budget of particles that reach the sea floor of which not all are buried.

Several methods are used to measure benthic fluxes. These include eddy correlation (Berg et al., 2003; Holtappels et al., 2013; McGinnis et al., 2008), gradient measurements in near-bottom water (Champanois and Borges, 2012; Holtappels et al., 2011; McGillis et al., 2011), high resolution profiling with sensors at the sediment-water interface (Glud et al., 2009; Lansard et al., 2008; Toussaint et al., 2014), vertical solute distributions in surficial sediment obtained from extracted pore water (Rutgers van der Loeff et al., 1984), core or mesocosm incubations of intact sediment in the laboratory (Ekeröth et al., 2016a; Hall et al., 1996;

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Karle et al., 2007), and benthic chamber incubations in situ (Berelson and Hammond, 1986; Dale et al., 2014; Donis et al., 2016; Glud et al., 2016; Hall et al., 1989; Jahnke and Christiansen, 1989; Sayles and Dickinson, 1991; Smith Jr. et al., 1976; Sommer et al., 2009; Sommer et al., 2017). Despite the technical challenges, in situ investigations are considered to provide more accurate and reliable insights into benthic solute dynamics and are thus preferable compared to ex situ studies (e.g. Glud et al., 1994; Hall et al., 2007; Sundby et al., 1986; Tengberg et al., 1995; Witbaard et al., 2000).

In situ chamber incubations are normally either diver- or ROV-operated or carried out using automated stationary (lander) or mobile benthic platforms (crawlers). Incubations of sediment and overlying water to determine benthic fluxes have the advantage of being able to take water samples over time in parallel to sensor data, enabling quantification of additional important solute fluxes using post-deployment sample analysis. In addition, chambers can be exposed to various experimental treatments, e.g., injection of solutes or labelled organic material and chemical tracers (e.g. De Brabandere et al., 2015; Ishida et al., 2013; Nielsen and Glud, 1996; Sweetman and Witte, 2008). Despite the constant advancement of lander technology, the use of in situ chambers can still be seen as an invasive method due to the isolation of the chamber from the surroundings, i.e., water exchange and organic matter supply are impeded. For longer deployments this may prove to be an issue because the internal chamber conditions will not be the same as the external environment. For this reason, incubations normally last for short time periods, hours to days.

Using fieldwork conducted with the Gothenburg autonomous benthic lander systems as examples, this paper focuses on describing and summarizing experimental possibilities and methods to obtain high data reliability and quality during in situ incubation experiments. This is an important aspect within the field that, to our knowledge, has not been described since the benthic lander review published by Tengberg et al. (1995) 25 years ago. We also give examples of even more advanced flux measurements that are now possible with this equipment, recommendations to the benthic chamber lander community and for researchers planning to construct, modify and use benthic chamber landers. We start by describing the Gothenburg benthic lander systems and modifications

that have been made over the years to ensure high quality incubations. We then discuss different types of experiment that can be performed with these lander systems. Finally, we give advice and recommendations for procedures when working with benthic chamber landers.

2. The Gothenburg benthic lander systems

The fleet of Gothenburg benthic lander systems consist of three lander platforms: the minilander, the small lander and the big lander. This paper focuses primarily on the big (carrying four modules) and the small (carrying two modules) landers and their chambers. The big lander can be deployed autonomously and consists of an inner and an outer frame. The outer frame serves as the carrier platform for the inner frame, chambers and sensors. The main components of the outer frame are syntactic foam buoyancy packages, railway track ballasts that are released at recovery, a dual acoustic release system for the ballast release and underwater positioning, and surface tracking systems (flash light, VHF and satellite beacons). A multi-sensor logger can also be added for background measurements of e.g. currents, turbidity, salinity, oxygen, waves and water level (Fig. 1).

The two-frame solution has been developed for the big lander allowing for the flexibility to deploy only the inner-frame from small vessels. Both frames on the big lander are built from non-corrosive materials (titanium and various plastics). The big lander can carry 1–4 experimental modules, for example planar optodes (Glud et al., 2001; Glud et al., 2005) and incubation chambers, which can be exchanged as desired. In shallow waters the inner frame can be gently lowered and recovered with a rope attached to a surface float. Close to the inner frame we attach floats to the rope to keep the lander upright and to obtain the right buoyancy for an appropriate chamber penetration into the sediment (typically 10 kg of negative buoyancy per chamber in soft sediments and 30 kg in harder sediments). In soft sediments a “wooden collar” (Fig. 1, left), a sheet of marine plywood with holes for chambers, is mounted underneath the inner frame to avoid the frame and chambers sinking too deep into the sediment.

If deploying a lander in areas with strong currents, drag on the rope and on the surface float might pull the lander. In this case we attached

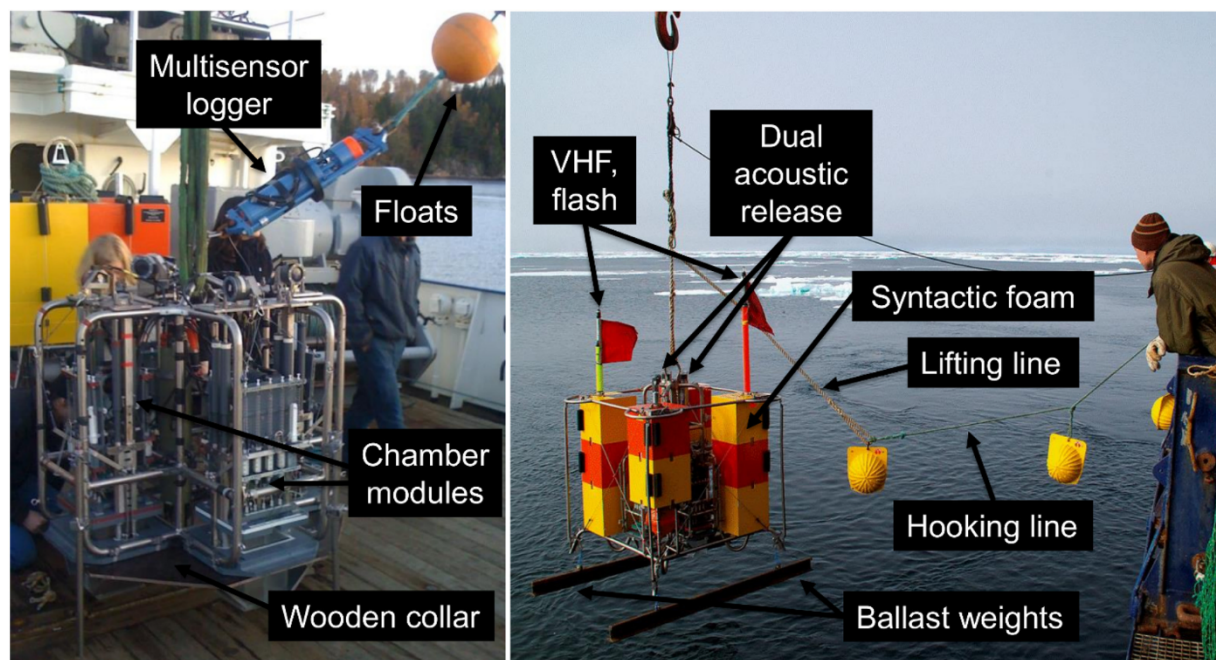


Fig. 1. Left: Inner frame of the big Gothenburg lander ready for deployment in shallow water with syringes and chambers armed. During deployment a multisensor logger is suspended above the incubation frame to record background information on currents, waves, salinity, temperature and oxygen. Right: Deployment of the big Gothenburg lander in autonomous mode with the outer carrier frame in the Arctic Ocean.

the rope and the surface float directly to an additional weight on the bottom. This weight is in turn attached to the lander frame by a rope lying on the bottom. In this way the lander frame is decoupled from the drag on the rope going to the surface. The deepest deployments that we have made with the inner frame on a rope have been about 200 m. In areas with high ship traffic this method could be risky as ships might sail over the surface buoy and the rope could get caught in propellers.

The present versions of the Gothenburg landers described in this paper were designed with a focus on flexibility, ease of operation for non-electronic/programming experts, fast turn-around time between deployments, low power consumption, and high quality control abilities. The landers have been deployed 308 times between 2006 and 2020 during 51 research expeditions in water depths ranging from 5 m to 5600 m. The number of successful chamber incubations has been 897 out of 988 (91% success rate). For a summary of measurements and experiments performed with the Gothenburg landers, and publications generated from these studies, see Table 1.

The small lander does not have a carrier platform. It consists of a smaller two-chamber frame. It is only deployed in shallow water, less than 200 m, on a rope.

2.1. Benthic chamber modules

The use of incubation chambers on landers to measure benthic fluxes of e.g. oxygen, dissolved inorganic carbon and nutrients has been common practice for over four decades. The basic incubation principle

Table 1

List of scientific publications, and their main focus, in which data obtained with the Gothenburg lander systems have been used.

Resuspension (N, P, C, Si, oxygen, Fe, Mn)	Tengberg et al. (2003) Almroth et al. (2009) Almroth-Rosell et al. (2012) Niemi et al. (2018)
Oxygen, respiration, planar optode	Glud et al. (2001) Ståhl et al. (2004a) Ståhl et al. (2004b) Cathalot et al. (2012)
Benthic C cycle	Nilsson et al. (2019) Rabouille et al. (2001) Ståhl et al. (2004a) Ståhl et al. (2004b) Ståhl et al. (2004c)
Benthic nutrient cycling (N, P, Si)	Brunnegård et al. (2004) Viktorsson et al. (2012) Viktorsson et al. (2013a) Viktorsson et al. (2013b) Ragueneau et al. (2001) Tallberg et al. (2017) Ekeröth et al. (2016a) Ekeröth et al. (2016b) Hall et al. (2017)
Benthic N cycle, $^{15}\text{NO}_3^-$ injections in situ	De Brabandere et al. (2015) Bonaglia et al. (2017)
Metals	Pakhomova et al. (2007) van de Velde et al. (2019)
Modelling utilizing benthic fluxes measured with the landers	Kiirikki et al. (2006) Stigebrandt et al. (2014) Almroth-Rosell et al. (2015) Hall et al. (2007)
Technology	Viollier et al. (2003)
Hydrodynamics	Tengberg et al. (2004)
Core recovery artifacts	Tengberg et al. (2005)
Sensors	Atamanchuk et al. (2014)
Ecological engineering (the By Fjord)	Stigebrandt et al. (2015)

of the Gothenburg lander chambers is similar to the first experiments of this kind performed by Pamatmat and Fenton (1968) and Smith Jr. et al. (1976).

A chamber module from the Gothenburg lander is shown in Fig. 2. The chamber walls are made of transparent polycarbonate, which is highly resistant to mechanical stress. The chamber has a 20 × 20 cm (400 cm²) square bottom area and 35 cm high walls with rounded corners (maximal chamber volume 14 L). On top of the chamber is a ventilation lid that can be opened and closed during the incubation. To prevent stagnation and the development of concentration gradients within the chamber, the incubated water is carefully stirred with a horizontally placed paddle wheel (Fig. 2). The paddle wheel is connected via a driving belt to a DC motor which is placed in a kerosene (lamp oil) filled PVC pressure case, pressure tested to 6000 m depth. The stirring speed can be adjusted and the paddles can be exchanged, depending on sediment conditions and the targeted hydrodynamic conditions. Ten 60 mL syringes (typically plastic syringes, Codan art. n. 628426 syringe 60 mL Luer-Lock) are attached on specially designed racks directly above the chamber (Fig. 2). Each syringe is connected to the chamber lid through a piece of Tygon® tubing with an additional volume of about 0.5 mL. The syringes are spring-charged and can be used for injection or water sampling. Stepper motors in PVC cases with rubber membranes (6000 m pressure tested and filled with silicon oil) trigger mechanical actions such as inner frame release, syringe injection/sampling, lid closing and opening and spring-activated sediment sampling.

The chamber modules (Fig. 2) on the Gothenburg landers have been carefully assessed with respect to hydrodynamic properties and inter-calibrated with other chamber designs (Tengberg et al., 2004; Tengberg et al., 2005). They have also been modified to make it possible to study the effects of resuspension on e.g. carbon turnover, respiration, and nutrient and metal fluxes (see Table 1). Other improvements have been to include long-term stable sensors in the chambers, for example turbidity sensors (Tengberg et al., 2003), oxygen optodes (Tengberg et al., 2006), salinity sensors and pCO₂ optodes (Atamanchuk et al., 2014). Recently we also successfully performed pilot tests including a pressure sensor to detect exactly when syringes are triggered and how long it takes for them to draw water from the chamber (results shown below).

2.2. Chamber sensors and electronics

Sensors and data-loggers used on the Gothenburg landers are from Aanderaa Data Instruments (<http://www.aanderaa.com>). The electronics controlling all mechanical functions such as lid closing, water/sediment sampling, and stirring motors have recently been updated to use an Atmega328p microprocessor and the free/open-source Arduino IDE software for firmware development. The DC motor control is implemented using an Atmega PWM output and a MOSFET high-side driver per motor. The stepper motor control is implemented using an MC3479 stepper driver chip pre-set to a clockwise rotation and full step mode. In this way the microprocessor only needs to enable stepper power and produce a step signal to run a motor (and trigger syringe sampling or another action). Step signal is common to all stepper motors, with stepper power control being implemented using an individual high-side MOSFET driver per stepper motor via 74HC595 shift register chips. The communication works over an RS232 cable connection (converted to TTL levels for interfacing with the Atmega microprocessor) using a simple plain text-based protocol. This makes fully manual operation possible using a serial terminal program or via a custom user interface software which greatly reduces lander preparation time and implements extra checks on the created sequence before uploading. A chamber lander (two landers in our case: the big lander, 4 chambers, and the small lander, 2 chambers) with such simple and reliable electrical circuits and proper user interface software can be easily operated by one person who is not an expert in electronic

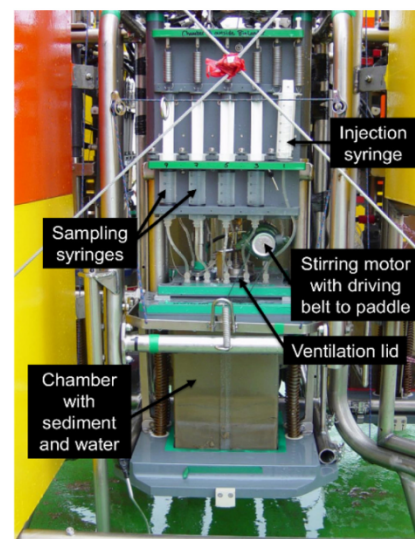
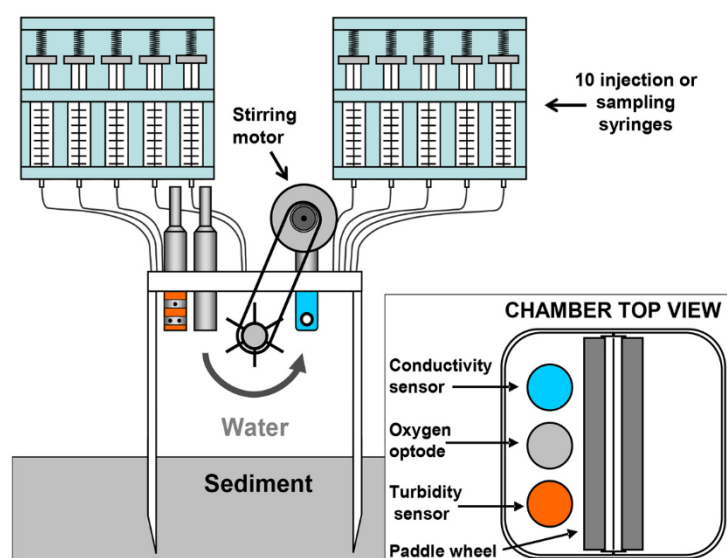


Fig. 2. Left: A drawing of the chambers used on the Gothenburg landers. Right: A photo of a chamber after big lander recovery with sediment and overlying water in the chamber, and syringes that have been triggered.

engineering, plus one or two people taking care of sampling from the syringes.

The total power consumption of the big lander is approximately 3 W when it is equipped with four chamber modules with stirring motors, 40 syringes for water sampling or injection (see below), sediment sampling and 15 sensors measuring every minute. By far the most power consuming components are the DC stirring motors in each chamber, which are operated continuously and consume about 0.5 W each. With a main alkaline battery pack of 864 Wh, the big lander can be operated for up to 14 days (5–10 deployments) before batteries have to be changed. In cold waters (0–4 °C) the battery capacity is expected to decrease by about 50%. The small lander has two chamber modules and runs both motor control and sensor measurements on a single 7-Ah rechargeable lead battery. This provides a maximal operation time of around 60 h.

2.3. Typical deployment scenario

Before every expedition, each chamber module with syringes attached is cleaned. The module is removed from the frame (this action takes about 5 min per module) and the entire chamber and water sampling system is carefully washed with phosphorus-free liquid dishwashing detergent, and repeatedly rinsed with tap and deionized water. In some cases, e.g. when targeting to measure trace metal and/or low nutrient fluxes, or when chambers are coming directly from workshop maintenance, the modules are acid washed. They are then submerged in a 0.2 M hydrochloric acid (HCl) bath for 24 h, followed by a deionized water bath for at least 24 h.

2.3.1. Deployment and first pre-incubation

When the lander with outer frame is deployed from the ship it descends by gravity at a rate of $\sim 40 \text{ m min}^{-1}$. At the sea floor, only the two ballast weights, which hang in ropes below the lander (Fig. 1, right), will hit the bottom and the outer frame stops 25 cm above the ballast. The inner frame with incubation chambers attached to the outer frame with a hydraulic piston and a “spinnaker hook” hangs about 50 cm above the sea floor. During descent and after landing the stirring is maintained, and both the ventilation lid at the top of the chambers and the bottom of the chambers remain open. This is done to ensure that any surface water or air bubbles potentially trapped by the chambers at the sea surface are removed, and that the sediment cloud which may be created by the bow wave and the impact at landing has time to clear or settle. This first (and

second, see below) pre-incubation procedure is made to ensure ambient bottom water conditions are established inside the chambers before the incubations are started. The total duration of the pre-incubation periods depends on the environmental conditions. E.g. in anoxic settings, these may need to be longer.

2.3.2. Second pre-incubation and incubation

Two hours after the landers reach the seafloor, the opening of the “spinnaker hook” is triggered by a stepper motor, and the inner frame falls by gravity to the sea-floor. The descent speed is reduced by the water-filled hydraulic piston to approximately $3\text{--}6 \text{ cm s}^{-1}$, so the chambers are gently inserted into the sediment leaving around 10–30 cm of overlying water inside the chambers (incubated water volume 4–12 L) to start the second pre-incubation stage. The penetration of chambers into the sediment can be controlled to some extent. If sediments are soft extra buoyancy floats can be added and chambers can be moved higher up in the inner frame. The stirring is continuous and the ventilation lids remain open to establish ambient bottom water conditions inside the chambers during this second pre-incubation period. Two hours after this second pre-incubation step is initiated, the ventilation lids are closed and the incubations are started. The length of the incubations typically varies from around 10 h in reactive anoxic sediments to $>70 \text{ h}$ in oligotrophic deep-sea sediments. If only oxygen uptake by reactive sediments is measured, the incubation time can be as short as 3 h.

Inside each chamber an oxygen optode monitors the oxygen concentration, a turbidity sensor measures the level of suspended particles and a conductivity sensor (with conductivity converted to salinity) enables the calculation of incubated water volume and verification that the chamber is not leaking (see below). These sensors are typically set to measure at 1 min intervals. On some deployments, pCO_2 optodes have also been installed in the chambers (Atamanchuk et al., 2014). During the deployment, the ten syringes attached to each chamber are activated automatically at pre-programmed times. The first and sometimes the second syringe are used to inject distilled water (to calculate incubated water volume from salinity change), tracers (e.g. ^{15}N -labelled nitrate; $^{15}\text{NO}_3^-$) or suspended material (e.g. algae or marl). The remaining eight or nine syringes are used to withdraw 60 mL samples from the chambers at set time intervals. When samples are withdrawn, the water is replaced with an equal volume of ambient bottom water, which enters the chamber from outside through a 1.5 mm inner-diameter and 400 mm

long diffusion barrier stainless steel tube.

2.3.3. Sediment sampling and recovery

At the end of the incubation once all syringes have been triggered, the chamber bottoms are closed to retain and bring up the incubated sediment with the lander at recovery. This provides the possibility for further sampling, e.g. for fauna, pigments, grain size, water content, etc. in the same incubated sediment. The construction and functioning of the chamber sediment retrieval device resembles that of a box-corer (e.g. Blomqvist et al., 2015), with two scoops, although the chamber bottom cannot be sealed as firmly. As a result, very soft or very stiff sediments cannot be reliably retained by our chamber sediment sampling mechanism. Sediment sampling is performed by releasing the energy saved in four springs per chamber, creating a total force of around 200 kg (2000 N) when the springs are compressed. The operation is dampened by hydraulic pistons filled with seawater, one piston per spring. However, the action of sediment sampling is quite sudden and the mechanism may produce mechanical shock that resuspends sediment into the overlying water during retrieval. Due to this, the quality of the lander-recovered sediments and overlying water, as observed through the transparent polycarbonate walls, is often considered insufficient to perform subsampling for porewater extraction and additional box-coring or multicoring may be required at some stations to assess sediment characteristics. Other experiments that demand high quality sediment sampling include e.g. trace injection experiments (e.g. Bonaglia et al., 2017; De Brabandere et al., 2015). Other autonomous incubation chamber landers that sample the sediments with better quality use slow motion shutters activated by motor or hydraulic power (e.g. Jahnke and Christiansen, 1989; Sommer et al., 2017).

To start lander recovery, an acoustic signal is sent from the surface to the lander acoustic release device. The Niskin bottle on the lander's outer frame is then closed to collect a background water sample and the ballast weights are released. The Gothenburg big lander has three independent systems for weight release, of which the first two are acoustic releases from iXblue (<https://www.ixblue.com/products/acoustic-releases>). In the worst-case scenario, if the acoustic releases fail, the third system to ensure eventual lander recovery is to wait until the four custom-made 20 mm diameter magnesium bolts, inserted into the ballast weights corrode, break and release the lander from the sea floor. To date we have never been forced to wait for this final safety mechanism and consequently we do not know exactly how long the corrosion of the bolts will take. The time needed for rupture depends on surrounding conditions such as temperature, currents, salinity and oxygen (or redox conditions). Typically, one set of magnesium bolts can be used for 2–3 deployments in the Baltic Sea before they are replaced with new ones. The smaller miniland system capable of carrying one experimental module has one acoustic release and one set of magnesium bolts.

For visual recovery at the surface, the landers have several systems that are activated by pressure switches at about 10 m below the surface. These systems include blinking flashlights, a VHF radio transmitter and a satellite beacon. To retrieve the lander at the surface from the ship and lift it on-board, float lines with low drag, ribbed glass floats are used (see Fig. 1, right).

2.3.4. Deployment quality control, sampling and preparation for new deployments

Two experienced persons can prepare the four-chamber big lander for a new deployment in 4–6 h while the two-chamber small lander can be prepared in 2–3 h. For both landers, our post-recovery checks and preparations for a new deployment are as follows in order of priority:

- After recovery note if the stirring motors are running and ventilation lids are closed. If stirring failed, it is important to check the sensor incubation data to find out when the stirring stopped. Samples taken after the stirring has failed may be disregarded due to likely stagnation of water in chambers. If ventilation lids failed to close,

samples may be disregarded due to unwanted exchange of chamber water with ambient water.

- Check that all syringes triggered, filled and if there are any gas/air bubbles in the syringes (which may affect later gas measurements) or sediment particles in the syringes, (indicating particle resuspension inside the chambers). Check that sediment was collected in the chambers and if overlying water remains over the collected sediment.
- Download and check sensor data and battery power:
- Check salinity readings for any chamber leakage (see examples below), and make a first quality control by comparing oxygen readings from inside chambers with those made outside (there should be a steady decrease of oxygen inside the chambers and no coupling between variations inside and outside chambers);
- Take samples from syringes for solutes (e.g. nutrients, metals, gases, DIC). This process can be particularly time-consuming due to a large number of samples (up to 9 samples per chamber and up to 6 chambers);
- Plan the next lander deployment;
- Prepare and mechanically arm chambers for new incubations and sediment sampling. Dispose of unused sediment and rinse chambers. This step also requires considerable time due to the need to ensure numerous components are armed and functioning. To save time, this mechanical preparation is usually carried out by one member of the research team in parallel to others working with syringe sampling.
- Arm the acoustic ballast release system. Place ballast weights under the lander and attach them to the lander with magnesium bolts and short ropes;
- Arm syringe racks, carefully noting the actual volume of tracer solution or Milli-Q water in the injection syringes. Attach syringe racks to the chamber modules and connect the tubing from syringes to the chamber lids;
- Upload the software configuration controlling all mechanical actions needed for the new deployment. When stirring motors start rotating it indicates that the lander is up and running, and ready to be deployed.

The time to prepare a lander for a new deployment can be reduced to 1–2 h if syringe racks and batteries are replaced with spare ones between deployments.

2.4. Determination of chamber volume and control of mixing, leakage and syringe sampling using salinity and pressure sensors

It is important to know whether there has been any water exchange between the incubated water and the bottom water outside the chambers (i.e. leakage). If leakage has occurred, the measured flux will be the net effect of the benthic flux and the water exchange and will not be representative of the sediment-water fluxes. It is also crucial to know the exact volume of the incubated water as e.g. a 10% error in the volume determination will thus result in a 10% error in the measured flux. In earlier chamber lander work, cameras and rulers (which do not take into account the variations in topography inside the chambers) have been used. Another frequent approach has been to inject a known amount of non-reactive tracer such as NaBr, RbBr, CsCl and ^{22}Na into the chambers (Tengberg et al., 1995), and to analyse the tracer concentration in a subsequent water sample from the chamber to estimate the chamber volume by simple dilution calculations. By monitoring the tracer concentration from samples over the whole incubation, it has also been possible to judge whether the chamber was leaking. This method takes considerable time due to tracer solutions needing to be prepared beforehand, and chamber water samples having to be collected and analysed for this specific purpose. The method also suffers from poor temporal resolution, and relatively large uncertainties associated with sampling and analytical errors. Another disadvantage is that the quality of the incubation will not be known until after samples have been

analysed, which is normally after the expedition, meaning that sample analysis from leaking (or otherwise malfunctioning) incubations is a wasted effort. Inside the chambers of the Gothenburg landers, we have mounted inductive conductivity/salinity/temperature sensors (model 3919 or 4319 from Aanderaa) that have a resolution of about 0.002 salinity and are calibrated for salinities 2–40. By injecting a known volume of distilled (Milli-Q) water or known concentration of salt water brine, a salinity change of 0.05–0.4 is registered. Being able to measure these salinity changes has the benefit of both acting as a leakage control and enabling the calculation of the incubated water volume. For higher accuracy of the injection volume, syringes are usually pre-calibrated by weighing before going to sea.

Equation 1. V_{ch} is chamber volume, S_1 is chamber salinity before injection; S_2 is chamber salinity after injection, V_{inj} is injected volume; S_{inj} is salinity of the injected solution.

$$V_{ch} = \frac{V_{inj}(S_1 - S_{inj})}{S_1 - S_2} \quad (1)$$

Prior to use in the field, this method was tested in the laboratory by filling chambers with a known volume of water and triggering multiple injections. The method was found to give a volume estimate with an absolute accuracy of approximately $1 \pm 4\%$ at salinity above 30 (Fig. 3). At lower salinities, the $(S_1 - S_2)$ term in the denominator becomes smaller and its relative error increases, resulting in errors in chamber volumes of $3 \pm 10\%$ at a salinity of around 7 (e.g. Baltic Sea). This method to determine the volume of the incubated water is reliable and easy to use. It has been applied in all chamber incubations (more than 700) performed with the Gothenburg benthic landers since 2007 on different stations with salinity between 2 and 35. Although this method does not take into account topographical variations inside the chambers it does allow an effective chamber height to be calculated from the precisely determined volume that can directly be used for flux calculations.

The mixing time in the chamber can also be determined if the sensors are set to log data at a faster interval. Mixing times were found to vary from seconds to hours depending on the chamber and stirring design (Tengberg et al., 2004; Tengberg et al., 2005). For the Gothenburg lander chambers the complete mixing time is normally below one minute (Fig. 3). In laboratory trials we also found this technique to work equally well for freshwater incubations by injecting higher salinity

water (data not shown). At stations where the bottom water temperature is below 0°C (e.g. the Arctic Ocean) there is a risk that Milli-Q water will freeze in the syringes. Under such conditions, we have successfully used injections of salt brine with a salinity of 70 and a lower freezing point. It is also important to note that the injection of water with a different ionic composition will not only change the salinity but also other components such as alkalinity.

Additional advantages of using conductivity sensors is that they offer reliable leakage control in the field at high temporal resolution (typically one-minute measurement interval on the landers, Fig. 4) and that unsuccessful incubations can be identified immediately after recovery. Consequently, if the data indicate leakage, samples will not be collected from the period affected by leakage. For an example of where leakage occurred at the end of incubations, see Fig. 4. As the salinity sensors are inductive, they are affected by other sensors and objects mounted close to them in the chamber lids. This gives a difference in salinity readings between sensors (Fig. 4 and Fig. 7) that can be corrected for by a simple cell factor correction (sensor manual, chapter 6, <https://www.aanderaa.com/media/pdfs/TD263-Conductivity-sensor-4319.pdf>). The calculation of chamber volume involves a salinity ratio, and the cell factor is cancelled out. Stability and quality control of the sensors are done with a resistor loop approximately once per year according to the manufacturer's recommendations. These sensors have been working reliably for >13 years and have not required any service or recalibrations.

The inductive conductivity/salinity sensors used on our landers have a borehole/ceramic tube (see the sensor specification sheet at <https://www.aanderaa.com/media/pdfs/conductivity-sensor-4319.pdf>) which must be properly rinsed and cleaned with a soft brush between deployments to avoid artifacts leading to lower readings. It is likely that such artifacts are created by either air-bubbles and/or sediment particle accumulation inside the tube, which will lower the conductivity and thus the sensor sensitivity. Dissolving air bubbles or dirt removal may introduce unwanted salinity dynamics which makes data interpretation more difficult.

A 6000 m-rated pressure sensor (model 4117F from Aanderaa) was recently installed in one of the Gothenburg big lander chambers to evaluate if measuring pressure inside the chambers could be an efficient method to detect mechanical action such as the closing of lids, timing of syringe sampling and injections. The sensor was logging at 2 s intervals and was able to clearly detect all mechanical events (Fig. 5). With this

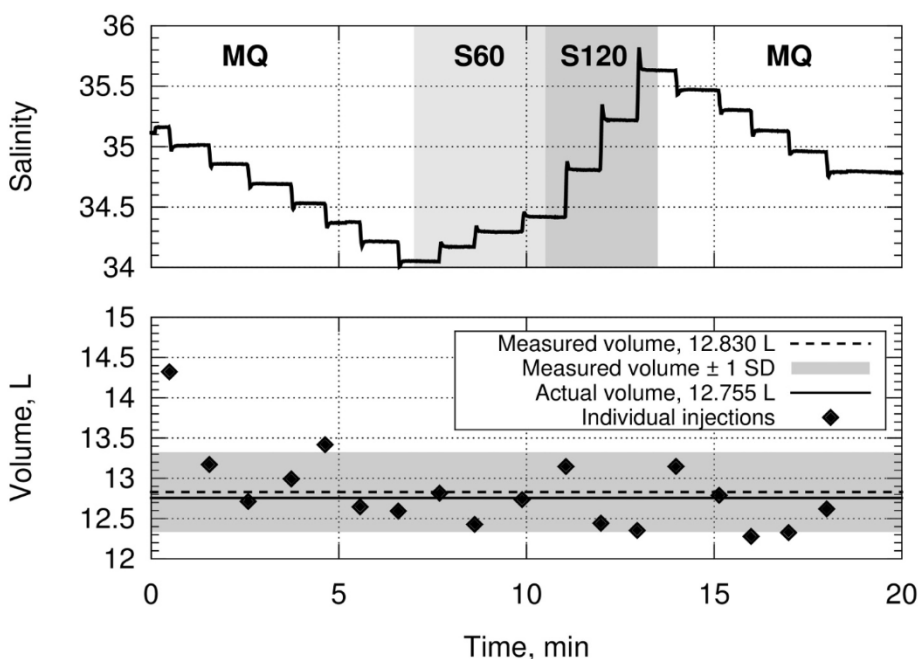


Fig. 3. Upper panel: Multiple 60 mL injections of Milli-Q water (salinity 0) and salt-brine (salinity 60 or 120). When injecting water, the same volume escapes the chamber through a tube, placed about 15 cm from the injection hole. The actual measured volume of the water that was filled into the chamber was 12.755 L. The average calculated volume from injections was 12.830 L. Lower panel: Individual volumes calculated from the injections. After each injection it took only a short amount of time for the salinity to become constant. This can be regarded as the mixing time. In these figures, salinity was measured every 2 s.

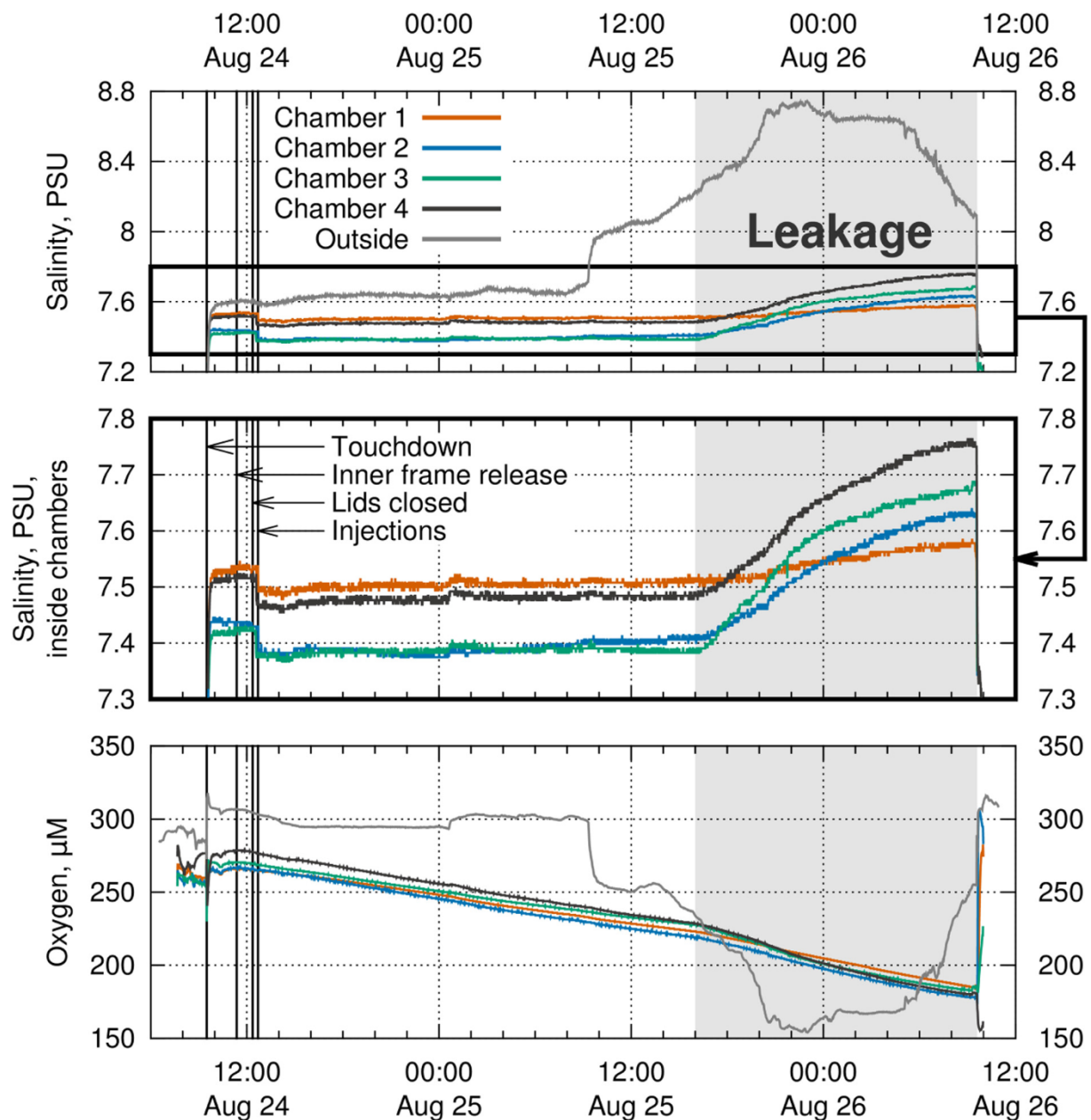


Fig. 4. Incubation examples from four parallel chamber incubations in the Baltic Sea. Due to strong bottom currents the lander moved and the chambers started to leak during the last third of the incubations. Water samples taken by syringes during this period were not analysed. Changes in salinity and oxygen in the chambers (top and bottom panel, respectively) were affected by changes of bottom water salinity and oxygen. The middle figure is zoomed in on the salinity inside the chambers and clearly shows the salinity change from the injection.

method, it is possible to confirm that the syringes take samples at the pre-programmed time points, which is important to controlling overall chamber function. In shallow water where wave action occurs, the noise levels of the pressure sensor are expected to increase which could potentially lead to difficulties in detecting small pressure changes while at greater water depths, the wave-induced noise will likely play an insignificant role.

2.5. Control of incubation functioning using oxygen sensors

The introduction of commercially available oxygen optodes in 2002 revolutionised oxygen measurements in oceanographic applications, including benthic chamber incubations. Advantages of this sensor, compared to electrochemical oxygen sensors, include long-term stability, better resolution, no stirring sensitivity, no irreversible pressure

effects (hysteresis) and no change in sensitivity upon exposure to hydrogen sulfide (H_2S) (Tengberg et al., 2006).

Stability and quality control of the oxygen optodes at air-saturation are carried out between expeditions and deployments by allowing the lander system to log data in air — in analogy with the methods recommended for saturation calibration of optodes on Argo floats and gliders (Bittig et al., 2014; Bittig et al., 2018; Johnson et al., 2015; Nicholson and Feen, 2017). Several optodes on our landers have not been calibrated since they were purchased in 2003, and they have been stored unprotected inside the chamber and acid washed before most expeditions. The optodes are always air saturation-checked between deployments and the zero-oxygen stability was verified at occasions when landers are deployed in anoxic H_2S -rich waters. The optodes have experienced minimal drift over the past five years. This is in agreement with what was recently reported in Bittig et al. (2018) where multiple

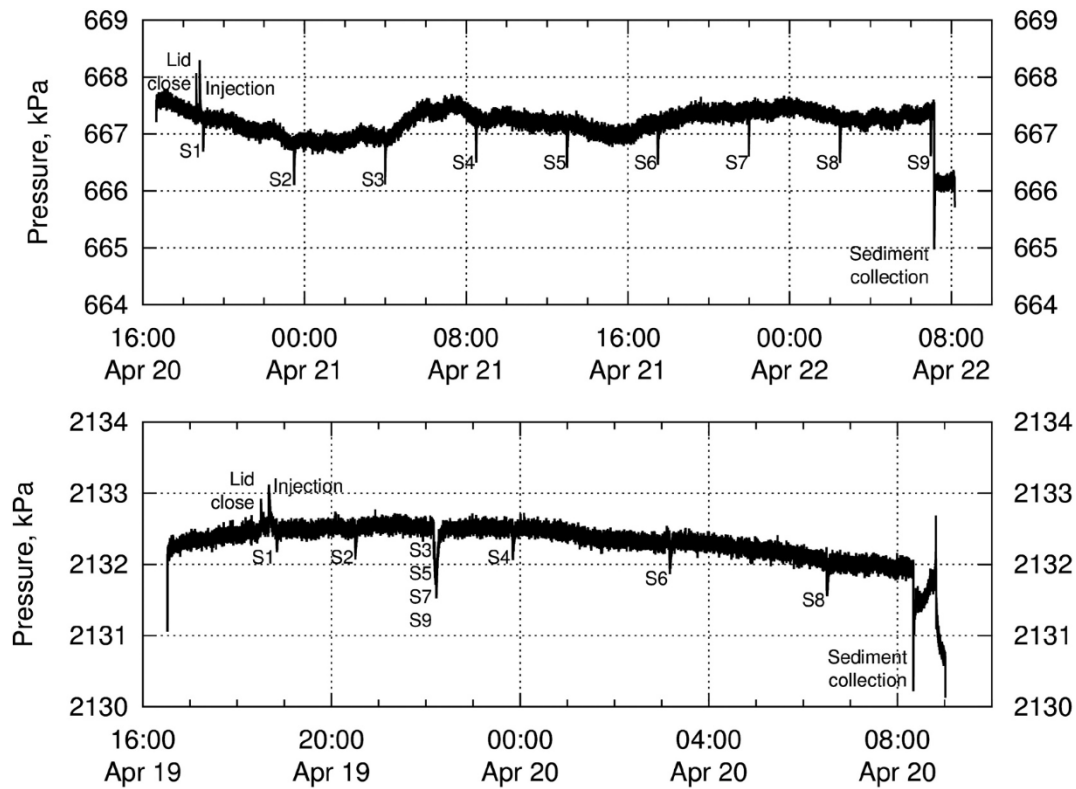


Fig. 5. Pressure variations recorded by a sensor mounted inside an incubation chamber during deployments at about 55 m (upper panel) and 203 m (lower panel) water depths in the Eastern Gotland Basin, Baltic Sea. The sensors gave feedback on water level changes and all mechanical actions like lid closing (0.6 kPa), syringe injection (0.8 kPa) and syringe sampling (-0.6 to -0.7 kPa). Syringe samples 1 to 9 are marked S1–S9. The lower panel shows an incubation in which four syringes sampled at the same time due to a programming error. This could be detected with the pressure sensor, and the calculation of an incorrect flux was avoided.

sensors were regularly multipoint-calibrated between deployments during time periods of up to seven years. Those sensors showed a maximum of 12% drift over 7 years, with the major part of the drift occurred during the first year and the sensors/foils became more stable with time. The drift of the zero point was small and thus could be compensated with a factor multiplication. We conclude that with the factor adjustment, the optodes (around 15) used on our landers since 2003 have always given high quality and consistent oxygen data.

On the Gothenburg landers, oxygen is measured with optodes both inside the chambers and in the ambient bottom water outside. When there is oxygen in the water of the chamber, oxygen measurements give an additional control of chamber functioning in parallel to the salt water/Milli-Q water injection (Fig. 4) and pressure sensor readings (Fig. 5). For example, if chamber stirring fails, it is easily detectable since the oxygen concentration (measured with the optode in the upper part of the chamber) will not decrease steadily anymore, as vertical concentration gradients will develop in the chamber (Fig. 9). If the oxygen concentration inside the chamber decreases by more than approximately $20 \mu\text{M}$ relative to the concentration outside, it is possible to detect when the chamber water is sampled, since small but instant positive spikes can be seen at the syringe sampling time points in the oxygen concentration (or oxygen flux) versus time graph. To be able to detect such small oxygen changes, faster logging (every 10 s) is required, in addition to a sensor resolution of about 10 nM and data filtering (moving average, Fig. 7). The first syringe sample of incubated water remains undetectable as the difference between chamber oxygen and ambient bottom water oxygen is too small at the beginning of incubations.

In environments with low or no bottom water oxygen, the oxygen measurements will also serve to ensure that the chamber ventilation/pre-incubation times at the bottom are sufficiently long so that i) the water of the chamber does not include oxygenated water and/or bubbles

from surface waters, and ii) any oxygen dissolved in and released from plastic parts (polycarbonate/PVC) of the chamber is ventilated out from the chambers. Gas (including oxygen) dissolution in plastic parts is a known feature that could create artifacts when carrying out incubations (e.g. Stevens, 1992) or when measuring low changes in respiration rates (e.g. Vikström et al., 2019; Wikner et al., 2013). We have found that a chamber ventilation time of 4 h with stirring and open lids is in general sufficient to not detect any contribution to chamber oxygen from plastic parts. When distilled water is injected for volume and leakage control (see above) in a chamber without oxygen, there will be a small, measurable increase in the chamber oxygen concentration. To minimize this effect, the distilled water can be boiled for at least 10 min and allowed to cool in a gas tight environment (e.g. glass bottle or metal container) to remove oxygen and other gases from solution. Alternatively, water can be vigorously bubbled with N_2 for at least 15 min to remove any oxygen.

When working in oxygenated environments, bottom-dwelling animals (benthic fauna) can be enclosed inside the chambers during incubations. Depending on the fauna present, their activities can have a significant influence on benthic fluxes of biogeochemical solutes. At such oxygenated stations it is always useful to be able to collect and sieve sediments in order to quantify and describe faunal assemblages' potential impacts on fluxes. Typically, in marine sediments not affected by hypoxia, the macrofauna contribution to the overall benthic respiration (measured as oxygen uptake) has been estimated to be 40–75% (Glud et al., 1994; Glud et al., 1998; Glud et al., 2003). The relative contribution of macrofauna becomes lower with water depth and seems to play an insignificant role in the deep sea (Glud, 2008).

Air bubbles may occasionally be trapped inside the chambers and carried down to the sea floor when the lander is deployed. In these cases, the observed oxygen flux describes the net effect of the bubble dissolution and sediment respiration rates. After bubble dissolution is over,

the flux may be then be affected by the increased concentration of oxygen inside the chamber (Fig. 6). A higher chamber oxygen concentration leads to a higher flux according to oxygen dynamic models (e.g. Hall et al., 1989). While we have observed the effect of bubble dissolution on chamber oxygen dynamics at shallow stations (<20 m), we have not yet encountered this effect at greater depths, most likely due to bubbles dissolving under pressure during the ventilation period on the seafloor. This observation further highlights the importance of including a proper initial ventilation phase at the sea floor prior to starting the incubations.

2.6. Calculation of benthic oxygen flux

The main purpose of oxygen measurement is to measure the benthic oxygen fluxes in different environments (examples shown in Fig. 7). Respiration by fauna and microorganisms in the sediments consumes oxygen and produces DIC as a product of organic matter degradation. In coastal sediments where oxygen uptake rates are relatively high, often 10–40 mmol m⁻² d⁻¹, 3–6 h of incubation is usually sufficient to obtain measurable oxygen uptake rates. Because of lower analytical precision from syringe samples (see below) longer incubation times of around 15–40 h are often needed to calculate fluxes of nutrients, metals etc. In oligotrophic and deep-sea sediments, incubation times of around three days may be needed to obtain significant, measurable fluxes from syringe samples (e.g. Ståhl et al., 2004c). When measuring respiration rates, and benthic fluxes in general, it is recommended to not allow the oxygen concentration decrease by more than 25–30% from the initial concentration within the incubation (Dalsgaard et al., 2000), since oxygen uptake rates are concentration-dependent and decrease with decreasing oxygen concentrations (for a further discussion of oxygen uptake kinetics, see e.g. Hall et al. (1989)). At low oxygen concentrations, sediment fauna can also be affected and oxygen uptake rates might thus alter significantly due to changes in faunal respiration or activity. Bioirrigation (water movement between burrows and overlying water by benthic infauna) can also be impacted by variations in oxygen availability, which may affect solute fluxes. Additionally, fluxes of redox sensitive elements such as P, N, Mn, Fe dramatically change when oxygen concentration decreases below certain level (Pakhomova et al., 2007; Severmann et al., 2010); an in situ determination of oxygen concentration where this has occurred is given in Fig. 11. However, longer incubations where oxygen levels are reduced to low values can be used to study changes of benthic reactions and processes in areas where bottom water oxygen frequently reaches hypoxia or anoxia.

Oxygen flux can be calculated in several ways, depending on the benthic conditions. The flux (J) is calculated as the slope of the oxygen concentration (C) versus time (t) curve, which is multiplied by the chamber volume and divided by the area. This is equivalent to multiplying the slope with the chamber height (H):

$$J = H \frac{dC}{dt} \quad (2)$$

The oxygen versus time curve can be virtually linear (Bouldin, 1968; Hall et al., 1989) (Fig. 4), which is the simplest case. The oxygen flux can then be calculated as independent of concentration or time, so one can use either a linear fit over a portion or all the data, or simply divide the oxygen concentration change by the incubation time. This is the most usual scenario observed at stations such as those with high oxygen concentration and low oxygen consumption rates. The chamber oxygen data can then be fitted with a straight line with an r^2 value above 0.99 (e.g. Fig. 4). The oxygen penetration depth (OPD) under these conditions is generally greater than 1 cm indicating a respiration (i.e. reaction) limited scenario. The slope of the oxygen versus time linear fit multiplied with chamber height gives the flux rate. In contrast, organic-rich accumulation bottom sediments with oxygenated or hypoxic bottom water show another type of dynamics (Figs. 6, 11). Here, the capacity of sediment to take up oxygen is high, the OPD is typically shorter than few mm, and oxygen flux and OPD decrease as oxygen inside the chamber is consumed. This transport-limited O₂ uptake and chamber oxygen data agree very well with the zero-order uptake model (Hall et al., 1989).

Oxygen uptake in some sediments with high bottom water oxygen concentrations have repeatedly exhibited unusual oxygen dynamics over the incubation time. At these stations, a high initial oxygen uptake rate decreases by a factor of 2–3 soon after the beginning of incubation, and stabilizes towards the end of the incubation (Fig. 8) which can be challenging to explain. A diagenetic oxygen consumption model would require a corresponding decrease in OPD, while non-steady state modelling shows a comparable (by magnitude) effect due to the closing of the ventilation lid. Assuming that the mechanical energy of stirring cannot dissipate from inside the chamber after the lid closes, the diffusive boundary layer (DBL) thickness should decrease more or less instantly. This decrease in DBL thickness produces a small “pulse” of oxygen into sediment, which takes time to propagate and dissipate in non-steady state conditions. However, at the measured OPD (1–2 cm; data from oxygen microprofiling in the laboratory; data not shown) the predicted effect of non-steady-state pulse is short-lived and cannot fully explain the time scale of oxygen flux changes (modelling data not shown). In this case, it can be difficult to decide what the correct representative oxygen flux value is. The initial decrease of oxygen may be an artefact, leading one to question what the true flux characteristics for the incubation are (as the same type of dynamics should affect samples used to calculate other solute fluxes), and what the true oxygen flux is. The former is probably the net change over the total time. For the latter we would propose to use the flux value at the end of incubation extrapolated using the Hall et al. (1989) model towards the initial oxygen concentration.

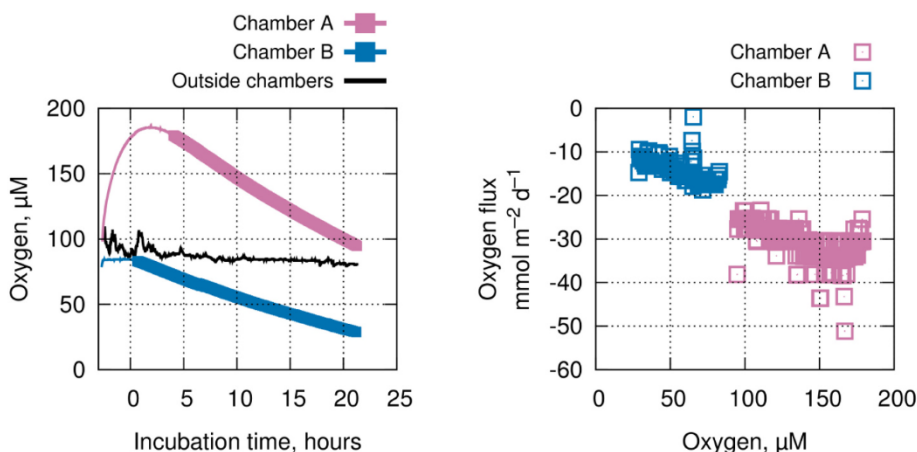


Fig. 6. Air bubble dissolution dynamics. The lid at chamber A (small lander) was prematurely closed so air bubbles were trapped and dissolved. Chamber B carried out a normal incubation (good ventilation, lid closed on time). Left panel: chamber oxygen concentration versus time. The thick line marks data selected for flux calculation. In chamber A, the initial oxygen concentration increase corresponds to the full dissolution of an air bubble of approximately 8 mL volume (normal pressure). Right panel: instantaneous oxygen flux value ($H \times dO_2/dt$, where H is chamber height calculated from chamber volume) versus oxygen concentration. Oxygen dynamics in both chambers appeared to follow the same flux versus oxygen concentration trend.

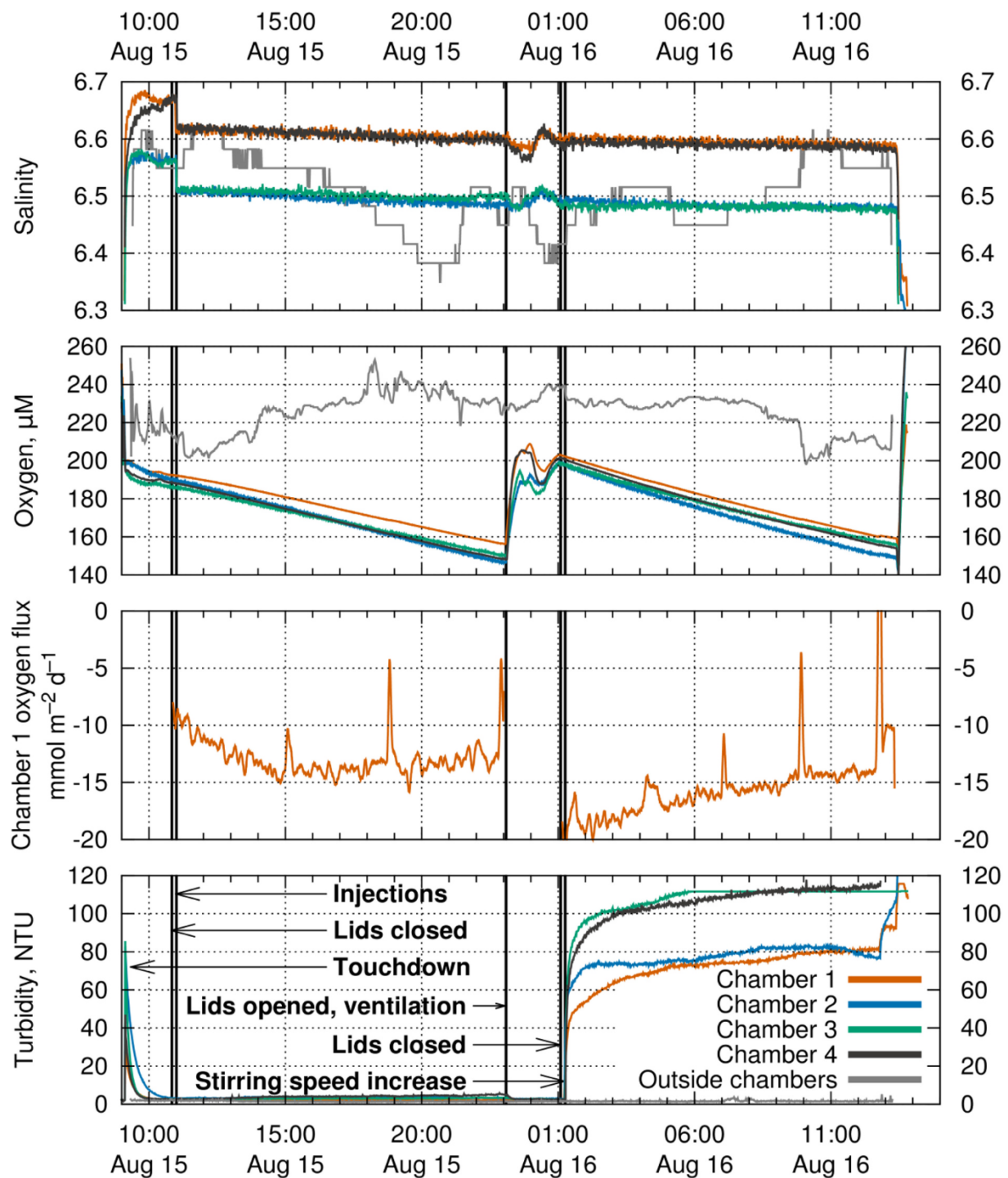


Fig. 7. Repeated incubations to study the effects of sediment resuspension in the Gulf of Finland, Baltic Sea (Niemistö et al., 2018). During the first half of the deployment, stirring was slower and no sediment resuspension was introduced which was reflected by low turbidity readings. At the start of the incubation, MQ-water was injected in order to calculate water volume and for leakage control. Halfway through the deployments, the chamber lids were automatically opened to ventilate the chambers and then closed again. High stirring was then introduced to study the effects of sediment resuspension, reflected in the higher turbidity readings. The third panel (oxygen flux) shows a change in the instant oxygen flux value, which is essentially dO_2/dt multiplied by the chamber height. Before plotting the derivative, these data were filtered with moving average to reduce the effects of sensor noise. Spikes in oxygen concentration are due to ambient water entering chambers to replace water sampled by syringes. The method of filtering noise from the oxygen data and plotting the instantaneous derivative amplifies changes in oxygen flux, making it possible to see when water samples were taken from the chambers, provided that the difference in ambient bottom water oxygen and chamber oxygen concentration is large enough.

Non-constant oxygen fluxes during chamber incubations may pose a problem when comparing to other solute fluxes determined by chemical analysis of the syringe water samples. While oxygen concentration can be monitored continuously with optodes, the number of chamber water

samples is limited to nine in our incubation chambers. In a situation where the oxygen concentration and flux change are important during incubation, one must select the adequate extent of oxygen data needed for the flux calculation. In the extreme case (Fig. 11) where oxygen

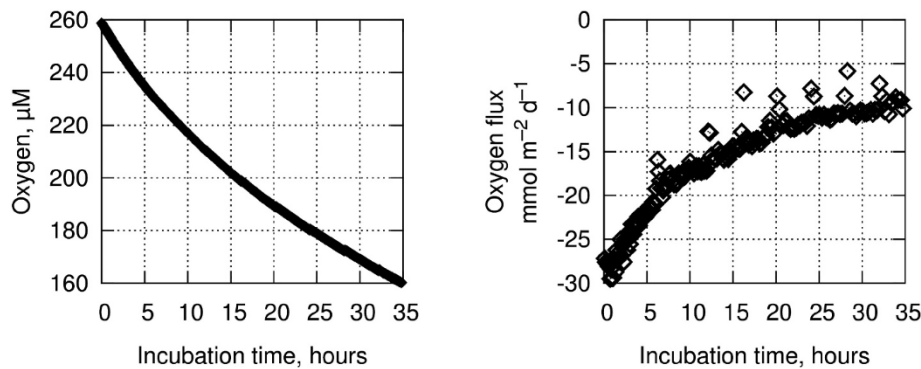


Fig. 8. Example from the northern Gulf of Bothnia at a water depth of 12 m of non-linear chamber oxygen dynamics in well oxygenated bottom water. In this extreme case oxygen uptake decreased from around 30 to 10 $\text{mmol m}^{-2} \text{d}^{-1}$. Similar dynamics was also observed at deeper stations in the Gulf of Bothnia.

becomes fully depleted, the oxygen consumption rate decreases from the initial value to zero. As explained above, it is also well known that the exchange dynamics of e.g. phosphate is affected when the water of the chamber becomes anoxic. Simultaneous measurements of oxygen (high resolution optode data) and phosphate (syringe samples) in a chamber can be used to determine at which oxygen concentration the phosphate flux starts to increase during oxygen depletion (Fig. 11).

Sommer et al. (2008) described an incubation chamber system which counteracted oxygen depletion by pumping the incubated water via 20 m of gas-permeable silicon tubing installed inside a larger oxygen reservoir chamber (also equipped with a mixing device). Their control system measured dissolved oxygen with optodes in ambient bottom water, inside the incubation chamber and inside the reservoir chamber. In this way the total oxygen inventory in the reservoir and incubation chamber could be monitored while total oxygen uptake was quantified and the oxygen concentration inside the incubation chamber at pre-set levels (70–75% of ambient oxygen level, for longer than 60 h). If simultaneous quantification of oxygen uptake inside the incubation chamber is not required (e.g. measured in parallel in a series of “normal” incubations), the gas-permeable tubing used to replenish oxygen in the incubated water can be mounted in ambient bottom water.

For most of our incubations carried out with the Gothenburg landers, we use the initial oxygen flux calculated from a linear fit of a small initial fraction of data points as a representative value of the oxygen uptake rate. To determine what range of the oxygen data to use in the calculations, we have used the maximal r^2 value (typically better than 0.99) as a criterion, i.e. the number of data points during a minimum of 30 min in the initial portion of an incubation that give the highest r^2 value. This approach cannot remove “events” such as effects of resuspension or syringe-filling; any such data must be removed beforehand. Because of this it is important that all oxygen data are manually inspected prior to performing the calculations.

2.7. Resuspension and turbidity

Turbidity is a key parameter when investigating sediment transport, planning dredging activity and assessing sediment resuspension. The two most common optical ways to quantify particles in suspension in natural seawater are measuring light attenuation (transmissometer) or reflection (optical backscatter sensors (e.g. Karageorgis et al., 2003)). We routinely use optical backscatter turbidity sensors inside and outside the incubation chambers to ensure that the levels of resuspension that may be created by the lander chambers during normal operation are low and not significantly different from natural levels outside the chambers. Turbidity data can additionally be used to check stirring quality. For example, noise in the turbidity data disappears when the stirring stops working, most likely due to larger particles quickly settling (Fig. 9, lower panel). This change in chamber turbidity signal can be seen before the oxygen consumption rate levels out as discussed in the previous section.

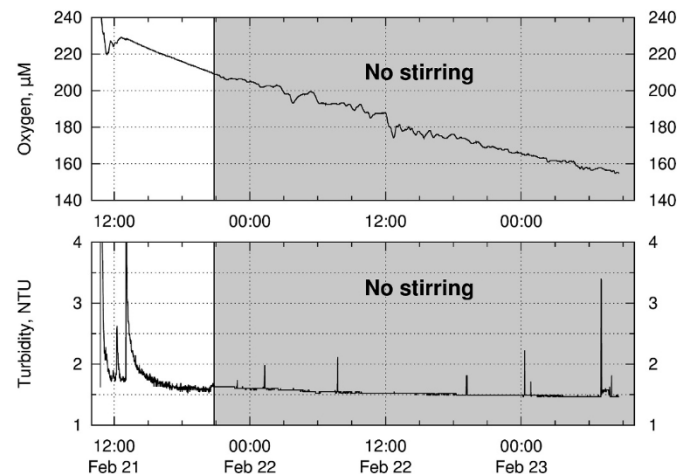


Fig. 9. Oxygen and turbidity data as means of stirring control. The stirring system was broken around 20:50 Feb 21 (2019). First strong spike (before 12:00 Feb 21) on the turbidity data corresponds to landing on the sea floor, the second strong spike (after 12:00 Feb 21) corresponds to injection. Data from the small lander, chamber B, Horsens fjord (Denmark), depth 10 m. Noise on the turbidity data disappears more or less immediately when stirring is broken. The turbidity response seems to appear slightly earlier when stirring stops working. Oxygen decrease changes from very smooth to somewhat irregular after chamber stirring was broken.

However, if the sediment is soft and populated by fauna, the bio-irrigation and bioturbation of infauna can increase turbidity levels. In this case, the change in chamber turbidity signal due to stirring failure can be difficult to detect and oxygen data is more likely to provide a better means of quality control.

Turbidity sensors on the Gothenburg landers are factory-calibrated with specific concentrations of silicon beads (Formacin) suspended in water, which result in sensor readings in e.g. nephelometric turbidity units (NTU). While a general conversion relation between NTU and suspended sediment concentration does not exist, specific calibration curves for each type of sensor, grain size and nature of the suspended material can be created in order to obtain an accurate transformation to suspended solids in mg L^{-1} . The turbidity data from the sensors on the lander can be transformed from NTU to mg L^{-1} suspended material by taking multiple water samples at each station, which are filtered and compared to the turbidity sensor readings according to descriptions given in (Tengberg et al., 2003).

The Gothenburg landers have been used in several projects aiming to characterize the effects of natural and man-made sediment resuspension on benthic solute exchange, e.g. in the Skagerrak, the Baltic Sea (Gulf of Finland) and in a Scottish sea loch (see list of publications in Table 1).

Longer continuous measurements, typically 10–12 months, with multi-parameter instruments (logging salinity, oxygen, temperature, currents and turbidity) 0.5–1 m above the seafloor assessed the occurrence of natural sediment resuspension events. This was followed by deployment of the landers for 24–48 h measurements in which incubations were done with and without introduction of resuspension (mimicking the natural resuspension events) through faster stirring of the chamber water (Fig. 7). In one example (Almroth-Rosell et al., 2012) the effects of man-made trawling/dredging and resuspension was simulated and studied by initiating the sediment collection system and thereby mechanically agitating the sediment inside the chamber at the seafloor. It has been difficult, however, to set targeted levels of resuspension in the chambers. The difficulties stem from hydrodynamic variations inside parallel chamber incubations, caused by differences in the chamber penetration depth, heterogeneity of the sediment structure, composition of the incubated sediment and local variations in the critical shear stress. We have found that even when the stirring speed is similar in four chambers, the level of sediment resuspension that is created can be quite different (Fig. 7). In Fig. 7 repeated incubations were carried out by automatically opening the lids and ventilating the chambers with ambient water halfway through the deployment. The first set of incubations was run without creating re-suspension inside the chambers. After ventilation the lids were closed, and the stirring speed increased to create and study sediment re-suspension.

2.8. Time series sampling of water from chambers: Calculation of fluxes and quality control

Benthic fluxes of various solutes are estimated from their concentration changes over time in the chambers during the incubation following the same idea as for oxygen flux measurements presented above but with sample-based data. The concentration data for each solute is, most commonly, fitted to a linear model estimating the concentration change versus time using least square regression, although non-linear models have sometimes been used (e.g. Forja and Gómez-Parra, 1998). Each chamber of the Gothenburg landers carries nine sampling syringes. Thus, for these landers, fluxes of solutes (requiring water sampling) are normally determined from nine data points. Flux estimates from other types of landers and ex situ incubations are typically based on a lower number of sampling points (Ekeroth et al., 2016a; Fuchsman et al., 2015; McManus et al., 1997). There are also numerous examples in the literature of benthic flux estimation by linear regression of only two samples (e.g. Tuominen et al., 1999; Villnäs et al., 2012). Using few samples for estimation of benthic fluxes provides low or zero statistical power in the evaluation of the achieved regression models. Another issue associated with using too few data points is that the common assumption of linearity with respect to the concentration change per time in the incubation unit cannot be assessed from the data (for an example of a non-linear nutrient flux, see Fig. 11). Using fewer data points also makes it more difficult to detect data outliers caused by, for example, contamination or analytical errors.

When calculating benthic fluxes a number of assumptions are made:

- All chemical processes (organic matter mineralization and other early diagenetic processes) are assumed to occur in the sediment and porewater. The dissolved mineralization products can diffuse into the overlying chamber water, while dissolved components from the overlying chamber water can diffuse into the sediment. Transformations in the overlying chamber water are assumed negligible. The chamber water is sampled and the concentration changes over time are used to calculate fluxes.
- Changes in concentrations are assumed to be the end result only of biogeochemical processes which take place in the sediment and porewater, and not due to advection or water exchange. Incubations on coarse-grained or highly porous sediments will result in fluxes that are the sum of mineralization and advective transport.
- Changes in concentrations are assumed not to affect the release/consumption rates, that is, linear concentration changes and constant flux rates are expected. In case of reactive sediments, running incubations for prolonged periods may deplete oxygen therefore the release of mineralization products becomes non-linear (concentration-dependent fluxes). In case of oligotrophic sediments the rates are slower so risk of running into non-linear dynamics is reduced.
- Stirring is designed to keep chamber water uniformly mixed so that no water stagnation occurs and no vertical concentration gradients occur in the chamber water. Chamber DBL thickness may be affected by the chamber stirring device, however the DBL thickness inside and outside the chamber is assumed to be the same. If this is not the case, the fluxes are assumed to be insensitive to possible DBL differences as demonstrated in Tengberg et al. (2005).
- Chambers completely block particle transport from overlying water and from the sides. The assumption is that over the period of the incubation the measured fluxes are independent of the isolation from natural particle fluxes.
- Concentrations of solutes in samples withdrawn from chamber water by the syringes are assumed to be stable. It is assumed that no change in concentrations occurs over the time sampled water is in the syringes, that there are no transformations between different species of the same element, that precipitation is not removing dissolved components etc. It is also commonly assumed that the syringe material and dissolved components do not interact which may not be true when gas is sampled using plastic syringes or silica is sampled using glass syringes.

Following recovery, water samples are usually filtered (except for gas sampling). It is also possible to attach a filter between each sampling syringe and the chamber, so the sampled water is filtered at the moment of sampling, however, the sampling mechanics must then be designed to overcome the extra resistance created by the filters. Tubing connecting the syringes to the chamber (and filters if mounted) can be left with air or filled with water and should be as short as possible to keep the air or water volume low. Both the presence of air and water saturated with oxygen can affect redox-sensitive species (e.g. oxidize and precipitate dissolved iron, or H_2S) and cause problems when sampling for dissolved gases. When filling tubing with water, the volume must be measured, and sample dilution must be taken into account.

The method for data evaluation and calculation of benthic fluxes from solute concentration time-series data measured with the Gothenburg lander chamber syringes has been continuously improved over the last decade. A description of the different methods used is given in Hylén et al. (in prep) where we suggest a method for the evaluation of benthic chamber incubation data in order to obtain objectively evaluated fluxes in a standardized way (Hylén et al., in prep). Accompanied by an easy-to-use R script (R Core Team 2017), the method also gives information about whether data fulfill the assumptions for linear regression: linearity in the relationship between dependent and independent variables, normally distributed errors, equal variance of residuals (homoscedasticity), and uncorrelated errors (e.g. Montgomery et al., 2012). The method can shortly be described as follows: Sample concentrations are first corrected for the small dilution that takes place in the chamber when new bottom water enters as samples are taken. At time t , the following dilution, Dil_t , is added to the measured concentration C_t :

$$Dil_t = V_s \frac{C_{t-1} - C_{bw}}{V_c} + Dil_{t-1} \quad (3)$$

where V_s is the syringe volume, V_c is the chamber volume and C_{bw} is the measured bottom water concentration. The dilution effect is generally so small that it does not change the flux. After the dilution correction, a linear or quadratic least-square regression line is fitted to data of sample concentrations versus time based on the corrected Akaike information criterion. Studentized deleted residual index and Cook's distance values

are calculated for each data point (Belsley et al., 1980; Cook and Weisberg, 1982; Williams, 1987). Certain threshold values are used to identify points deemed to have high leverage and influence which then go through a closer inspection and are potentially removed. Diagnostic graphs of data are used to ensure that the assumptions for linear regression are fulfilled. Finally, the flux is calculated by multiplying the chamber height with the initial slope of the regression line, i.e. the derivative of the line's equation at the first time point. Fluxes are considered to be significant if the p -value for the F-statistics is lower than 0.05.

When correcting the sample concentrations for dilution, it is necessary to know the chemical composition of the ambient bottom water. Sharp concentration gradients close to the sea floor often make it difficult to get representative data of solute concentrations in the water refilling the chamber from the Niskin bottle on top of the lander. Under such circumstances, unless the bottom water can be sampled very close to the sediment surface, the best option is to assume that the average chemical composition inside the chamber just after the incubation has started reflects that of the near bottom water. Thus, the average concentration in the first sampling syringe in all chambers, which is activated shortly (about 10 min) after the chamber lids close, is often the best available estimate of the chemical composition of the ambient bottom water flowing into the chambers through the diffusion-barrier tube to replace water sampled by the syringes.

2.9. Time series sampling of water from chambers: Solute dynamics and examples of new flux measurements

2.9.1. Hypoxia effects on dynamics of nutrients and dissolved inorganic carbon

Most in situ chamber incubation work has been focused on benthic cycling of oxygen, carbon, nitrogen, phosphorus and silicon (Table 1). Fig. 10 shows examples of dissolved inorganic carbon (DIC) and nutrient concentration time series during chamber incubations in the Eastern Gotland Basin, Baltic Sea, in 2015 (Hall et al., 2017). On this occasion, the lander was deployed at 210 m depth in a previously long-term anoxic

area. A few months before the sampling, a major Baltic inflow (e.g. Hall et al., 2017 and references therein) from the North Sea oxygenated the area and at the time of sampling the bottom water oxygen concentration reached $\sim 40 \mu\text{M}$. After re-evaluation according to the method described above, the fluxes were generally found to be slightly higher than stated in Hall et al. (2017). The sediment on which these incubations were carried out did not contain any fauna, however the variability in the measured fluxes was significant (Fig. 10) and probably reflects the small-scale spatial variability at this station reinforced by the oxygenation event. In particular DIP (Dissolved Inorganic Phosphorus) fluxes varied from a consumption of -0.11 to a release of $+0.17 \text{ mmol m}^{-2} \text{ day}^{-1}$. This type of situation has previously been encountered in sediments with hypoxic overlying water, where oxygen drops below a certain concentration at the sediment-water interface and the flux changes from a low efflux or an influx to a significant efflux; see also Fig. 11 where this was observed.

It is well known that benthic phosphate fluxes increase upon bottom water oxygen depletion. Results from a deployment with the big lander in the By Fjord (Swedish west coast) demonstrated that it is possible to determine in situ at which oxygen concentration in the overlying water the shift in phosphate flux occurs using a lander chamber (Fig. 11). Carrying out chamber incubations so that oxygen is depleted has thus proved to be a useful exercise to mimic process changes in areas where oxygen levels approach anoxia/hypoxia (e.g. Balzer et al., 1983; Sundby et al., 1986).

2.9.2. Metals

High quality in situ determinations of benthic fluxes of trace metals can be difficult to obtain, mainly because of low natural concentrations, a high risk of contamination during deployment, chamber incubation or sampling and loss of reduced metals through chemical oxidation. Mn and Fe fluxes have previously been successfully measured with our benthic landers (Pakhomova et al., 2007; van de Velde et al., 2019). With the purpose of showing that the deployment and retrieval procedures of our landers do not introduce trace metal contamination, we

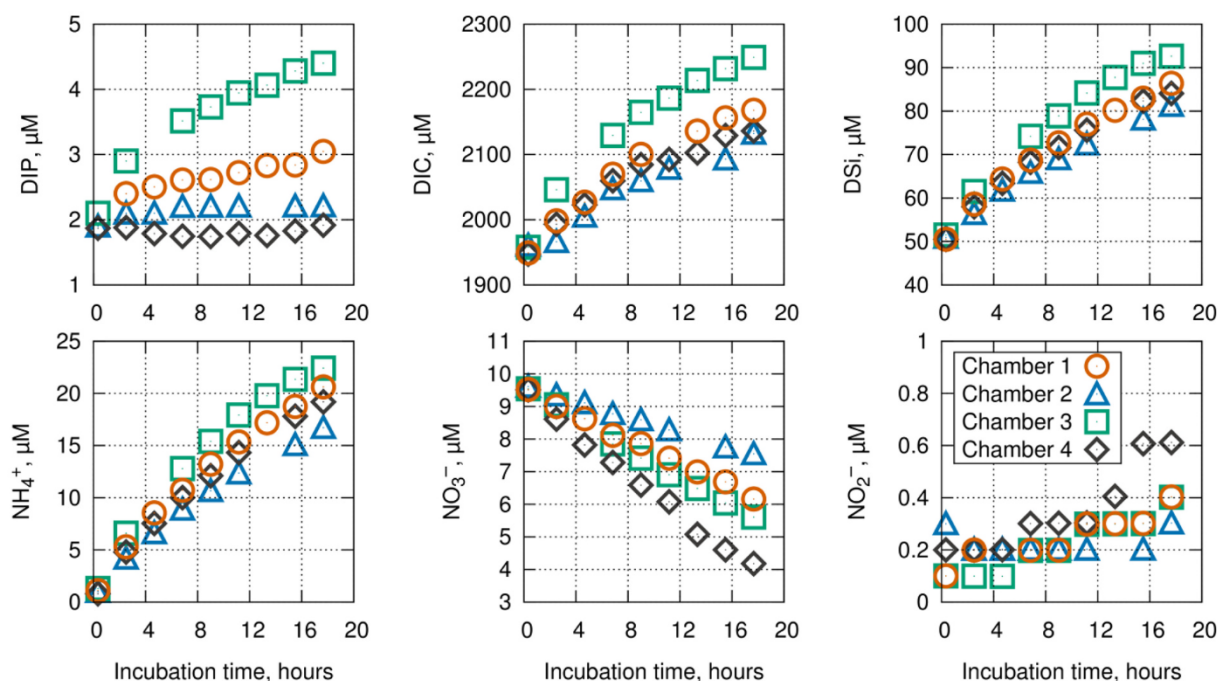


Fig. 10. Examples of the evolution of nutrient and DIC concentrations versus time in the chambers during a single deployment of the big lander at station F in the Eastern Gotland Basin, Baltic Sea, in early July 2015. Bottom depth 210 m. DIC: dissolved inorganic carbon; DIP: dissolved inorganic phosphorus; DSI: dissolved inorganic silicon. Calculated fluxes in $\text{mmol m}^{-2} \text{ day}^{-1}$ have the following range: DIP: -0.11 to $+0.17$; DIC: 35 to 112 ; DSI: 8.7 to 10.6 ; NH_4^+ : 4.7 to 8.0 ; NO_3^- : -0.4 to -1.0 ; NO_2^- : 0.0 to 0.08 , on the limit of the detection. The oxygen consumption rates ranged from O_2 : -10 to $-20 \text{ mmol m}^{-2} \text{ day}^{-1}$. Negative values mean uptake, positive mean release.

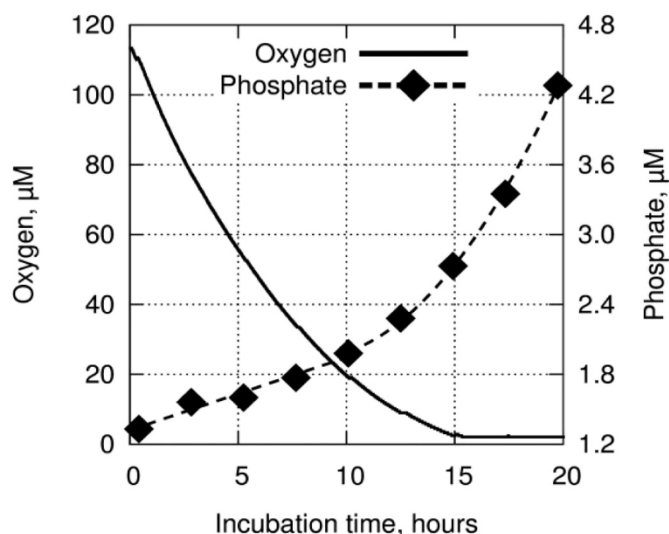


Fig. 11. Examples of the evolution of oxygen and phosphate concentrations versus time in a chamber of the big lander deployed in the deep part of the By Fjord. This lander deployment was made after the deep part of the By Fjord had been oxygenated by ecological engineering (Stigebrandt et al., 2015). Oxygen was measured at 1-min intervals with an optode. At a chamber oxygen concentration of about 20 μM , the phosphate flux dramatically increased.

recently equipped one chamber with a plastic “dummy bottom” to perform an in situ blank incubation where bottom water was physically isolated from sediment. In this paper we presented in situ flux measurements of five metals; manganese, important metal in early diagenetic cycling (Burdige, 1993); molybdenum, vanadium and uranium, trace metal proxies for paleo-redox conditions (Tribovillard et al., 2006), and arsenic, a highly toxic metalloid that is often a component of dumped chemical ammunition (Sharma and Sohn, 2009). During the

blank incubation, the concentrations in the water of the chamber stayed quasi constant (red triangles on Fig. 12), which suggests that no metals were released from the chamber walls or other parts of the lander. The other three chambers, where sediment was incubated as usual, showed an increase in metal concentrations in the water of the chambers. The concentration increase is thus consistent with a metal flux from the sediment of these redox sensitive species. The scatter on the As data was most likely caused by As analysis in sea water via mass spectrometry being difficult, due to spectral interference from ArCl^+ compounds (ICP-MS Interferenz Tabelle, Finnigan MAT, Bremen, 1995).

2.9.3. Methane

Benthic fluxes of methane can also be measured by in situ chamber incubations (Sommer et al., 2009). It is usually advisable to use glass syringes and Viton® tubings for such gas sampling, and to use incubation chambers built of glass or metal. It is also recommended to fill the tubing which connects the sampling syringes to the chambers with water to avoid dissolved gases equilibrating with the air space that remains in the tubing's or syringe's inner space. In these circumstances, the small dilution of the syringe water sample must be taken into account. However, in our experience, it is possible to run measurements of dissolved methane in situ with plastic chambers and sampling syringes. Fig. 13 shows examples of methane data from incubations of two sediments, one well oxygenated and one anoxic. In the well-oxygenated shallow sediment in an area with fish and mussel farming facilities (As Vig, Horsens Fjord, Denmark), methane concentration showed a clear increase from 16 to 24 nM in one chamber, and no trend in the other. In a deeper, stagnant and long-term anoxic fjord basin (By Fjord, Sweden) with sediment rich in organic material, the methane concentrations were on the order of a few μM . A strong linear release of methane enabled the demonstration of intense anaerobic mineralization processes in surface sediment.

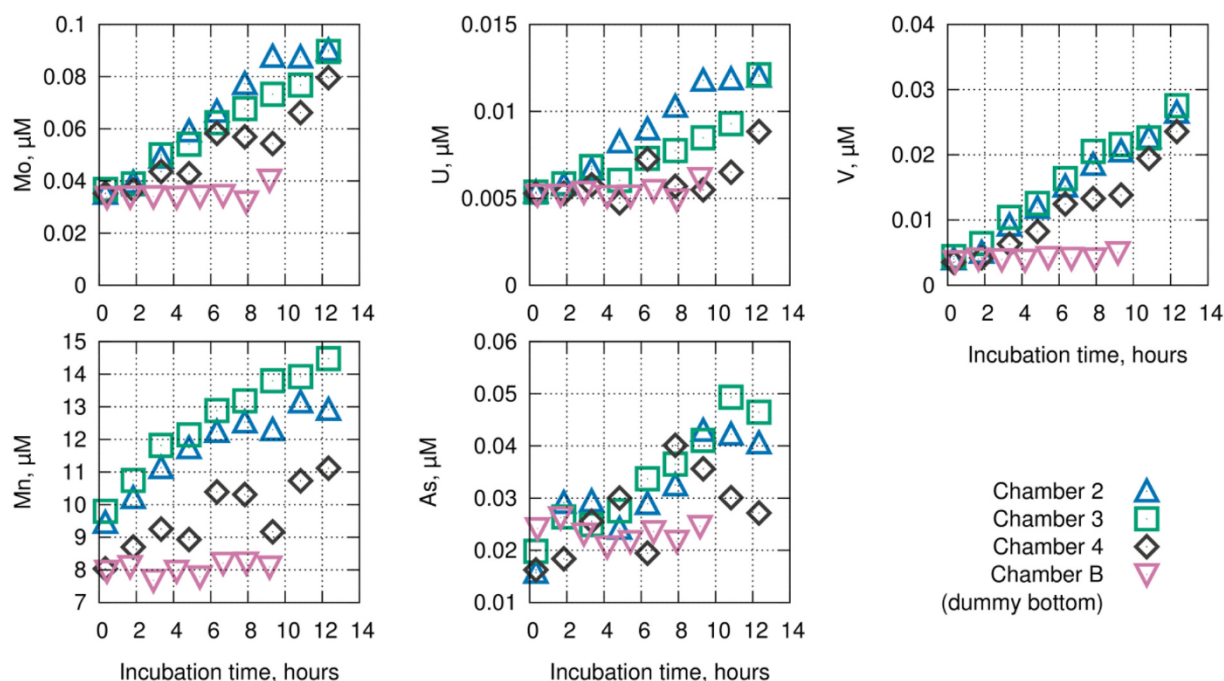


Fig. 12. Evolution of dissolved metal concentrations in the water of benthic chambers. The results are from lander deployments in the Eastern Gotland Basin, Baltic Sea, April 2017 at station E with a water depth of around 170 m. Chamber B was equipped with a dummy bottom and served as a “blank incubation” (i.e. incubation of bottom water isolated from sediment). The other chambers incubated sediment and overlying water. The bottom water had just returned to anoxic conditions (anoxic chamber water) after the 2014–2015 major Baltic inflow (see above). Description of the analytical determination of the metals is given in van de Velde et al. (2019).

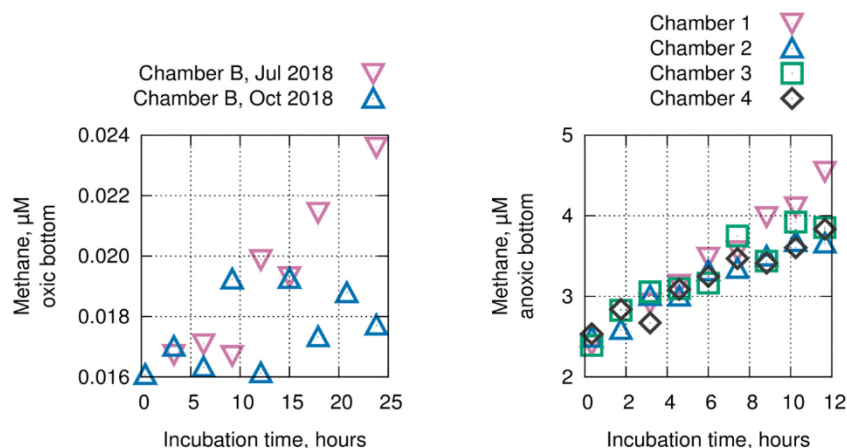


Fig. 13. Chamber methane concentrations versus time during incubations. Oxic station (left): As Vig, Horsens Fjord, Denmark. Depth 10 m, two incubations run during two different field campaigns in July and October 2018. Anoxic station (right): By Fjord, depth 35 m, long-term anoxic bottom, Big Lander, four parallel incubations (October 2009).

2.10. Injection experiments

Each of the 10 sampling syringes (per chamber) on the Gothenburg landers can easily be converted to inject a solution as opposed to taking a sample thus providing the opportunity to carry out a range of benthic manipulation experiments.

2.10.1. Nitrate reduction measurements

Denitrification and dissimilatory nitrate reduction to ammonium (DNRA) have been studied in situ in several experiments using the Gothenburg benthic landers (Table 1) where the stable isotope ^{15}N has

been used as a tracer. 10 min after the chamber lid has been closed, a sample is taken to measure the natural background concentration of nitrate. Ten minutes later, a $^{15}\text{NO}_3^-$ solution is injected into the chambers to reach a final concentration of 40–70 μM (depending on the chamber volume). Another sample is taken after ten minutes to elucidate the final enrichment concentration (i.e. total $\text{NO}_3^- = ^{14}\text{NO}_3^- + ^{15}\text{NO}_3^-$). The eight remaining samples are taken with 2–4 h intervals. This allows for the confirmation of linear ^{15}N -labelled nitrogen (N_2) production over a number of time points, a critical assumption to the use of the ^{15}N isotope pairing method (Nielsen, 1992; Risgaard-Petersen et al., 2003). After recovery of the lander, the samples are divided into aliquots and

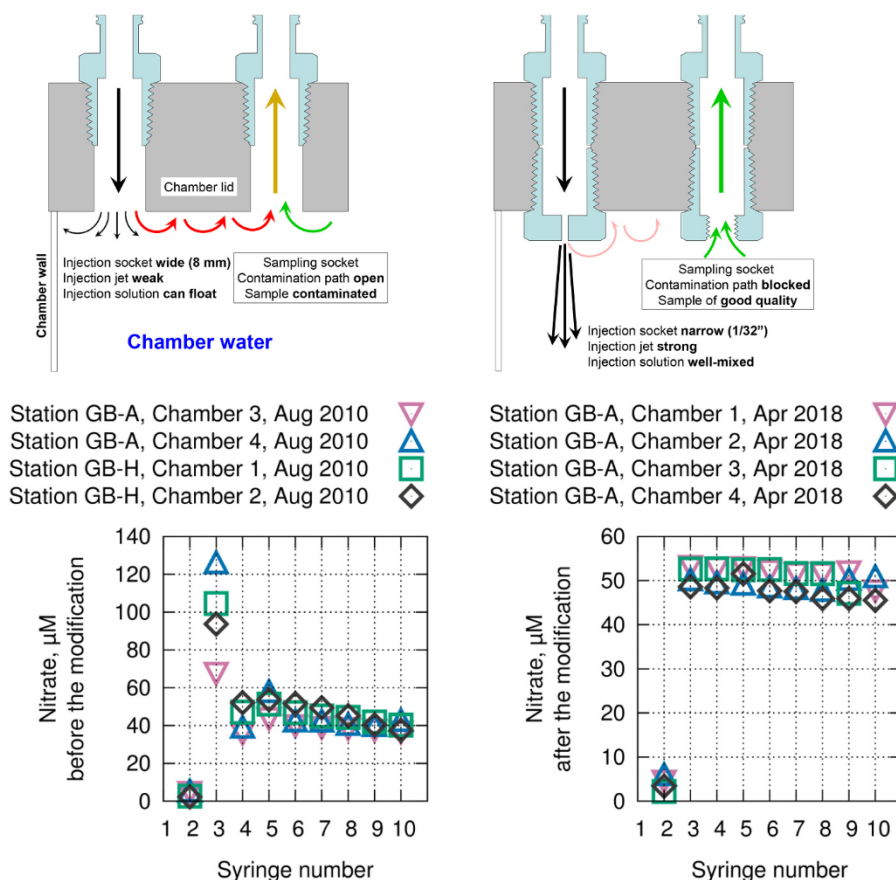


Fig. 14. Top panel, left: the original structure of chamber injection and sampling ports. Right: modification by adding adapters to avoid sample contamination by injecting solution. Bottom panel, left: $^{15}\text{NO}_3^-$ injection experiment, demonstration of syringe 3 and 5 sample contamination. Syringe 2 took a sample before the injection to measure the ambient bottom water NO_3^- level, and 10 min later syringe 1 injected the $^{15}\text{NO}_3^-$ solution. Syringes 3–10 took samples from the incubated water 10 min after the injection and then at regular intervals. Right: successful sampling after modification of syringe nozzles. The difference in NO_3^- concentrations between syringe 3 and 2 is used to estimate the concentration of $^{15}\text{NO}_3^-$. In case of contamination in syringe 3, the concentration of $^{15}\text{NO}_3^-$ after injection must be extrapolated based on the values from uncontaminated samples. All stations located in the Eastern Gotland Basin, Baltic Sea. Station GB-A was around 60 m deep, station GB-H was around 44 m deep and both were permanently oxygenated bottoms.

analysed for dissolved inorganic nitrogen concentrations and the isotopic compositions of the ^{15}N -labelled end products of NO_3^- reduction (N_2 , N_2O and NH_4^+) as described in De Brabandere et al. (2015) and Bonaglia et al. (2017). Since measurements of the concentrations of the ^{15}N -labelled products are performed in water samples, production rates of these species need to be corrected for the fraction of product that accumulates in the sediment. This is done by sampling incubated sediment that is retrieved with the lander, or, if no sediment is retrieved, in parallel whole core laboratory incubations (Bonaglia et al., 2017; De Brabandere et al., 2015).

One practical problem observed during the ^{15}N injection experiments was that some samples (syringe 3, sampled 10–20 min after the injection and sometimes syringe 5) were regularly found to contain concentrations of nitrate largely exceeding what was expected from the calculated dilution of injected ^{15}N -nitrate solution (Fig. 14). The cause of the problem was that the injection solution was MilliQ-water based with density lower than the incubated bottom water. To solve this problem, the inner side of the injection and sampling ports were modified. The injection holes were equipped with 1/32-in. nozzles producing strong injection jets, which can even slightly disturb and resuspend soft sediment surface (Fig. 9). The sampling holes were equipped with an adapter creating vertical spacing to the chamber lid. This solution should also be useful in cases when there is strong gas release from the sediment. Released gas will accumulate beneath the chamber lids resulting in gas being taken up by the syringes instead of chamber water. This can be avoided by installing longer inner sampling nozzles as a means to only sample water in the chambers. The corresponding loss of injected MilliQ water will lead to a systematic over-estimation of the chamber volume of about 1% (see section on the determination of chamber volume above). This artefact can also be overcome by the use of nozzles.

2.10.2. Phosphorus retention

In the Baltic Sea, high loads of phosphorus have resulted in serious and large-scale environmental problems such as eutrophication, extended areas with hypoxic/anoxic conditions, regular occurrence of toxic cyanobacteria blooms, and loss in amenity value. The small Gothenburg lander (carrying two chambers) was deployed to do in situ injection experiments on the capacity of marl (lime-rich clay) to bind phosphate (thus preventing it from reaching photic surface waters and fuelling the blooms). The experiment was conducted at 104 m depth in the coastal basin of Kanholmsfjärden, Stockholm archipelago, north-western Baltic Sea. At the beginning of the incubations, approximately 2.5 g of fine-grained ($<0.75\ \mu\text{m}$) marl suspended in 60 mL of deionized water was injected into each treatment chamber. Non-treated chambers were running in parallel. The results from this pilot experiment (Fig. 15) showed a reduction in phosphate flux from the sediment from $1.06 \pm 0.18\ \text{mmol m}^{-2}\ \text{d}^{-1}$ in control chambers (mean $\pm$ standard deviation, $n = 6$ (Ekeröth et al., 2016b)) to $0.60 \pm 0.04\ \text{mmol m}^{-2}\ \text{d}^{-1}$ in chambers with marl treatment ($n = 2$, Blomqvist et al., unpublished results).

3. Recommendations on the operation of benthic chamber landers

In situ incubations of sediment with overlying water provide valuable and consistent information about fluxes and processes at the sediment-water interface. In this paper, we have described experiences from use during the last 14 years and over 300 deployments with our benthic lander systems. We have presented recent advancements and modifications in the field and given examples of lander performance and how quality and reliability of chamber incubations can be assessed. Furthermore, we have presented a wide range of measurements and experiments that have been carried out with our landers. Our main goal has been to demonstrate the possibilities that these systems offer to measure solute fluxes and study processes at the sediment-water interface. Based on our experience, recommendations are given below for

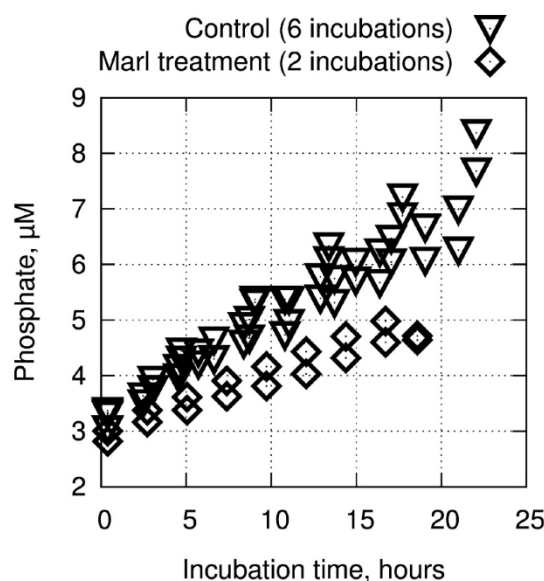


Fig. 15. Benthic effluxes of dissolved inorganic phosphorus (DIP or phosphate) at 104 m depth in the coastal basin Kanholmsfjärden, Stockholm archipelago. Fluxes were measured in situ with the small benthic lander under anoxic conditions.

using benthic chamber landers.

3.1. Chamber design

- Choice of materials.** It is convenient to have chambers built of polycarbonate. Polycarbonate is mechanically strong and transparent which is practical as enabling observation of incubated water and sediment on recovery: to check stirring, possible sediment resuspension, presence of and activities of trapped fauna (one trapped shrimp can alone resuspend the sediment more efficiently than our chamber stirring devices at the highest RPMs). Plastics are also easy to modify if more sensors are needed to be installed in a chamber. If the main purpose of a chamber system is to study gases, it is advisable to consider glass and metal for chamber construction and glass sampling syringes.
- Chamber ventilation lids** are very important to eliminate bubbles and surface water. It is necessary to ventilate the chambers long enough (for our chambers ca 4 h) before closing the ventilation lids to avoid artifacts from any trapped surface water and/or gas dissolved in plastic parts of the chambers diffusing into the chamber water. It is difficult to give generic recommendations for the ventilation time which depends on how the incubation chambers are designed and should be verified experimentally by the chamber user. Ventilation lids also make repeated incubations during a single deployment possible, by opening the lids for intermediate ventilation before closing them again to start ‘new’ incubations. For solutes that require sampling (e.g. nutrients, metals, gases), the number of chamber sampling syringes then limits how many repeated incubations that can be performed.
- Sampling/injection syringes** should be placed as close as possible to the incubation chambers to minimize the “dead-volume” of the tubing connecting syringes to the chamber lids. Some syringe types have double O-rings. It is recommended to cut the upper o-ring to avoid trapping air between the O-rings. If air is trapped, the pressure will compress the plastic syringe and it will not sample at depth.

- **Sampling and injection nozzles** in the lids should be designed to avoid unnecessary gas sampling or contamination of water samples by the injection solution. The injection nozzle should have a small diameter and protrude below the chamber lid.
- A “**diffusion barrier**” should be included on the chamber lid to let the ambient bottom water enter the chamber when syringes are sampling. A 1.5-mm inner-diameter, 400 mm long stainless gas chromatography tube is used on the Gothenburg landers.
- **Sampling of the incubated sediment** is recommended as it can provide valuable parallel information that can be used alongside flux data (e.g. fauna abundance). Landers with slow running sediment collection systems like a hydraulic scoop (Jahnke and Christiansen, 1989) or a motor operated shutter (Witte and Pfannkuche, 2000) generally collect high quality intact sediment with overlying water and are recommended.

3.2. Chamber sensor system

- For accurate chamber volume determination and leakage control, it is recommended to include high resolution **salinity** measurements both inside and outside chambers, and to inject a precisely known volume of Milli-Q or high salinity water at the beginning of the incubations. If an incubation chamber is leaking, measurement and water samples taken from that chamber should be discarded.
- **Turbidity** should be measured inside and outside chambers. Including turbidity sensors in the chambers is useful when doing resuspension experiments, and to examine if unexpected resuspension occurs, e.g. from landing, too high stirring speed, lid closing, sediment sampling or macrofauna activity. By collecting and filtering water samples at the site, a relation between the sensor readings (NTU and FTU) and the absolute suspended particle concentrations (mg L^{-1}) can be established.
- High sensitivity **pressure sensors** inside chambers are recommended to provide precise feedback on and timing of mechanical operations such as chamber insertion, lid closing, syringe triggering, sediment sampling and whether the lander moves during the deployment.
- **Oxygen optodes** inside and outside the chambers give robust and precise flux measurements when oxygen is present and offer quality control in low oxygen/anoxic environments. Measurements of oxygen are also useful for checking possible artifacts from any trapped surface water and/or gas dissolved in plastic parts of the chambers diffusing into the chamber water. The optodes used on our landers require minimal maintenance and have become more stable over time; they have drifted 7–15% over 15 years. An air saturation check of the optodes in air between deployments is recommended.
- As **sensor development** progresses towards compact, low power and reliable sensors, it is recommendable to include these in incubation chambers. Obvious candidates are sensors for pH, nitrate and pCO_2 , as well as for dissolved metals (e.g. Fe, Mn, Milani et al. (2015)).
- Including **measurements of currents** in the water surrounding the chambers can be useful to detect high currents which could cause the lander to move during incubation. A current meter can easily be mounted on the outer frame of the lander. Since modern current sensors normally also measure **tilt and heading**, they provide additional useful information on any lander movement during the deployment.

3.3. Planning and conducting measurements

- The Gothenburg landers can be deployed autonomously or on a rope. Autonomous deployment requires using a frame with buoyancy and a ballast release system which must be transported to the site together with enough ballast. The ship crane must be capable of lifting a heavy system and the ship must be large to accommodate the lander with the carrier frame on deck. If one plans to work in a shallow coastal area, it may be more practical to lower the lander on the sea floor with a rope. In this case only the frame carrying the chambers and electronics is needed, in addition to some floats, but no extra ballast. We have used this technique in areas with low currents and small waves down to 200 m water depth although deployments with a surface buoy can pose a risk in areas with ship traffic and/or fishing.
- It is crucial to have a good plan for recovery. Make sure that the weather permits the lander to be lifted up safely. If there is little local boat traffic, the recovery rope can generally safely be left attached to a surface buoy, otherwise it may be necessary to use an acoustic release with a rope canister or lay out a rope at the bottom for recovery by dragging. If there is intense fishing or bottom dredging activity at the study site, the lander may be dredged away and possibly damaged. When landers operate autonomously and leave ballast weights behind there can be occasions when it is necessary to know the precise location of surfacing, e.g. due to nearby ship traffic or ice coverage. Some ships are equipped with USBL underwater positioning systems which makes it possible to position the lander on the bottom and when it sinks or rises. The lander should of course additionally carry a transponder or an acoustic release with USBL capacity.
- The length of the incubation depends on both the sediment type and on the solutes that are measured. Measurements of oxygen respiration using chamber sensor data can be short (e.g., a few hours), as the sensors provide accurate and precise measurements at high temporal resolution. Development of well measurable signals from DIC or nutrient release based on samples normally takes longer. Oligotrophic sediments of the abyssal plains might require several days as the organic material is less reactive and mineralization processes are slow. For a low/intermediate oxygen flux of $5\text{--}10 \text{ mmol m}^{-2} \text{ d}^{-1}$ we usually run incubations for minimum 30–36 h and try to ensure that the in situ chamber volume as small as practically possible by making the chambers penetrate deeper into sediment. If oxygen fluxes are $15\text{--}30 \text{ mmol m}^{-2} \text{ d}^{-1}$, we do not incubate sediment for longer than 24 h to avoid excessive oxygen depletion. Making the pre-incubation period longer or incubated water volume bigger is also a possibility if one needs to deploy for a longer for logistical reasons.
- It is always difficult to decide on how to run experiments when one comes to a site which has not been studied before and there is little background information available. One way to assess how long lander incubations should be is to deploy the landers for a short (~ 3 h) pilot measurement of oxic respiration with optodes, without sampling the incubated water. After recovery, one can quickly evaluate oxygen flux, assuming that in situ volumes from chamber conductivity measurements are immediately available. It is then possible to adjust chamber height if needed and decide how long the incubations with water sampling should be to get proper flux measurements from chamber water samples without too large oxygen depletion (ca 25% max). When the bottom water is anoxic, this method cannot be used. However, anoxic bottom water often suggests relatively stagnant bottom water, intensive accumulation of organic matter and mineralization processes capable of quickly consuming the dissolved oxygen. In these cases, incubations may not need to be longer than 10–15 h.

- It can be useful to check existing monitoring data and/or to measure a CTD profile before each deployment which may provide enough insight into how long the incubations should be. Bottom water oxygen concentration, possible density stratification, water stagnation (or vice versa, properly ventilated bottom water due to strong currents) are all factors that can affect flux rates. Properly ventilated transport and erosion bottoms are usually a sign of stiffer sediment, lower organic material net deposition rates and lower benthic flux rates which possibly suggests longer incubation times and/or smaller incubation volumes. Better understanding of combinations of all these factors comes with experience.
- It is always preferable to directly sample sediment with a boxcorer to check sediment stiffness, color, the presence of a hydrogen sulfide smell or hard inclusions, such as stones, gravel, nodules or mussel shells, before a lander deployment. It is difficult or near impossible to run incubations on hard sediment, and presence of larger hard objects can cause the chambers to leak thus reducing probability of successful incubations. It is possible to avoid areas with very hard sediment using acoustic reflection measurements reported from the ship sonar. Very sandy sediments are also difficult to incubate due to sand being heavy and stiff and thus it is difficult for chambers to be inserted firmly and deeply enough (unless chambers are equipped with a special insertion mechanism like described by Janssen et al., 2005), and because sand can be permeable and cause chamber leakage, Janssen et al. (2005). Global databases on geological sediment properties (like EMODNET-Geology, <https://www.emodnet-geology.eu/>) provide important larger-scale data on sediment properties and composition which can be used to help selecting experimental sites where landers can be deployed successfully.
- An acid wash of chambers and syringes prior to expeditions is recommended for measurements of low nutrient and trace metals fluxes, e.g. manganese, molybdenum, vanadium, uranium and arsenic. We have good experience from using a 0.2 M HCl bath for 24 h followed by a distilled or deionized water bath for 24 h. Keeping each chamber in a plastic bag prior to deployment is important to prevent contamination during transport.
- There has been a long discussion about how large and how many chambers a lander should have. Our experience is that running 2 to 6 chambers in parallel allows to get statistically significant data on many stations in one week, or by running several different types of incubations at fewer stations or over a shorter period. A larger chamber gives more representative data on heterogeneous sediments, implying that the scale of heterogeneity is smaller than the chamber size. From a practical point of view we suggest having two or more chambers to compensate for possible chamber failure.

4. Remaining challenges

- Technology for controlling chamber penetration depth without pre-knowledge / assumptions of sediment stiffness;
- Controlled resuspension to pre-defined levels with feedback systems adjusting the stirring;
- Methods to keep oxygen concentrations or pH constant at pre-defined levels (e.g., for the determination of solute fluxes that would require longer-term incubations or for experimental work on hypoxia and ocean acidification);
- Chambers / approaches for flux studies in hard-bottom ecosystems;
- Chamber approaches that allow representation of natural advective exchange conditions for flux measurements in permeable media (e.g. subtidal sands);
- Compact, reliable, stable and energy efficient sensors for e.g. nutrients, pCO₂, pH, methane, hydrogen sulfide and dissolved trace elements (such as Fe and Mn);

- Possibility to measure mineralization processes in the bottom water in parallel with sediment-water incubations.

Declaration of Competing Interest

None.

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