Contribution of boulder reef habitats to oxygen dynamics of a shallow estuary

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HIGHLIGHTS

- Oxygen dynamics of a estuarine reef was similar to a nearby non-vegetated habitat.
- Eutrophic conditions caused pelagic processes to dominate oxygen variability.
- Benthic microalgae had similar primary production as macroalgae on reef.
- High benthic oxygen demand during a heat wave caused anoxia and net heterotrophy.

GRAPHICAL ABSTRACT

The oxygen dynamics of a shallow estuary is controlled by a series of uptake, release and exchange processes. At shallow depth, benthic flora and fauna associated with boulders contribute significantly to ecosystem primary production and respiration, but with increasing depth, pelagic processes becomes dominating.
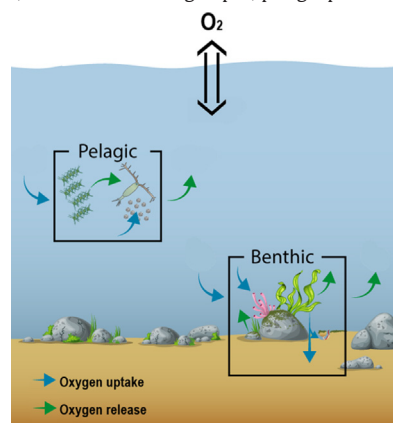
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ABSTRACT

We assessed the importance of boulder reefs to the oxygen dynamics of a shallow estuary during two growing seasons in 2017 and 2018. Using open-system diel oxygen measurements and benthic and pelagic incubations, we evaluated the relative contribution of pelagic and benthic habitats to the ecosystem metabolism along a depth gradient in two areas, with (Reef) and without (Bare) boulder reefs in the Limfjorden, Denmark. System integrated areal rates of gross primary production (GPP) and ecosystem respiration (ER) both increased with depth in both areas. Benthic contribution to system GPP and ER was highest at shallow depth where it represented 47 and 53% respectively. However, with increasing depth pelagic processes dominated GPP and ER (98 and 94%) even in the Reef area. Although the Reef area had higher biomass of auto- and heterotrophic organisms (macroalgae and macrofauna), benthic GPP was at similar level in both areas, due to a significant contribution from micro-phytobenthic organisms. The Reef area had lower sediment pools of organic matter, nitrogen and phosphorous and was slightly more oxygenated compared to the nearby Bare area. Extreme temperatures and higher levels of nutrients in 2018 caused a marked increase in benthic ER rates resulting in net heterotrophy (NEM = GPP − ER < 0) in 2018 compared to net autotrophy (NEM > 0) in 2017. Under current eutrophic conditions, boulder reefs do not contribute positively to the oxygen dynamics in the estuary. Reoccurring blooms of phytoplankton with high organic matter decomposition combined with high temperatures and dominance of

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fauna stimulate depletion of oxygen around the reefs. Significant improvements in water clarity are needed to regrow perennial macroalgae and induce net autotrophy. Under current turbid conditions, it is only recommended to establish boulder reefs in shallow (<3 m) parts of the estuary.

1. Introduction

Boulder reefs are very productive habitats, which under favorable environmental conditions can establish a high coverage of macroalgae. They extend from shallow to deep waters, range in size and compactness and are active sites for marine carbon sequestration (Krause-Jensen and Duarte, 2016). Therefore, boulder reefs can be considered as biological hot-spots for macroalgae and benthic animals, which serve as the base of the food web species (Stenberg and Kristensen, 2015), and encompassing a large range of sizes and compactness. In Danish waters, boulder reefs have for decades been diminished through the removal of stones to be used for port mills and construction works. An estimation from the Danish Nature Agency suggests that in the last 50 years around 55 km^2 of stones were removed from the Danish coastal waters, equivalent to 15,000–75,000 ton ash free dry weight of macroalgae (Helmig et al., 2020). Removal of boulders was mostly from shallow waters (<10 m), until it was banned by Danish laws in 2010. Although there was no systematic survey to estimate to what extent the removal of natural stone reefs affected the ecosystem, it has caused a decline in the abundance of associated macroalgae and fauna, and suggested have lowered water quality and primary production of benthic macroalgae (Krause-Jensen et al., 2012). In addition, significant amounts of stones are each year removed from the Danish estuaries because of mussel dredging (Nielsen et al., 2020). The Limfjorden is the largest estuary in Denmark which has changed from a pristine environment in the early 20th century, to a very eutrophic state in the 1980s. Since then the fjord has undergone many serious nutrients reduction efforts by the national authorities (Krause-Jensen et al., 2012), however, still remains in a poor environmental state (Hansen and Høgslund, 2021). Encouraged by a modelling study (Møhlenberg et al., 2008), a project was established to investigate the extent to which boulder reefs oxygenate sediments, reducing release of nutrients, making reefs a possible supplementary tool in water planning actions in accordance with the Water Framework Directive (2000/60/EC). This study was part of that project, and results obtained from an intensive research program carried between year 2017 and 2018 in two areas of the Limfjorden are presented here.

As boulder reefs host great diversity and biomass of autotrophic and heterotrophic organisms, areas dominated by such reefs are expected to be active sites for carbon and nutrients recycling, with a more variable oxygen (O_2) dynamics related to elevated primary production and respiration compared to similar shallow waters without reef structures (Krause-Jensen et al., 2015). Availability of light, suitable substrate, temperature, salinity and physical exposure, controls the abundance of macroalgae and animals (Dahl et al., 2003). In the subtidal shallow waters below the highly exposed zone, where sufficient light reaches the bottom, autotrophic organisms, like macroalgae, are usually dominant on hard structures, where they grow into several layers comprising an accumulated coverage of up to 300% (Carstensen and Dahl, 2019). In the very shallow zone, benthic primary producers are similarly key to sustain sea food webs (Christianen et al., 2017). As the water depth increases and light availability decreases, macroalgae thins out and the filtrating sessile benthic fauna usually becomes dominating (Dahl et al., 2003; Dahl et al., 2005; Dahl et al., 2020). In the Limfjorden, historical removal of boulders along with several decades of eutrophication has gradually changed the sediment substrate towards a higher organic content and finer particles (Krause-Jensen et al., 2011), which are more prone to resuspension further reducing light availability for growth at surface of benthic algae. The increased input of labile organic matter to the sediment, due to enhanced phytoplankton growth, induces a higher consumption of oxygen in the sediment, which combined with high temperature and stratification of the water column, favors development of hypoxic/anoxic events. This puts the biodiversity in high danger, especially perennial macroalgal species, which has decreased dramatically in the last decades in many regions (Wernberg et al., 2019).

Primary production and respiration are the major metabolic processes contributing to the carbon cycling in aquatic environments (Staehr and Sand-Jensen, 2007; Staehr et al., 2010). The balance between gross primary production (GPP) and ecosystem respiration (ER) determines the oxygen balance and the net ecosystem metabolism (NEM) as net heterotrophic (ER > GPP) or net autotrophic (GPP > ER) (Cole et al., 2000). Nutrient concentrations, light temperature and other factors have strong influence on seasonal patterns in primary production and respiration, causing shifts between autotrophic and heterotrophic states (Hoellein et al., 2013; Murrell et al., 2018; Staehr et al., 2018). Contributions from pelagic and benthic primary production and respiration to the whole ecosystem metabolism depend also on several factors (Bogert et al., 2007). Eutrophication, for example, can limit the light reaching the benthic area, by increasing pelagic productivity and phytoplankton biomass (Riemann et al., 2016). This reduces the relative contribution of the benthic primary production to the overall system metabolism, through the domination of pelagic processes. Benthic metabolism can dominate in shallow aquatic ecosystems (*e.g.* Bogert et al., 2007) with increasing level primary production in systems dominated by benthic vegetation (Krause-Jensen et al., 2012) stimulating net autotrophy (Dalsgaard, 2003; Staehr et al., 2018). The overall aim of this study was accordingly to understand how benthic and pelagic compartments contribute to the overall systems primary production and respiration rates, and if the boulder reefs significantly contribute towards improving oxygen conditions in the shallow eutrophic estuary, comparing an area dominated by boulder reef with a bare area dominated by soft sediments and gravel.

In this study, we used a similar approach as Staehr et al. (2018), with high frequency oxygen measurements in the open water, pelagic and benthic habitats, to evaluate the relative contribution of benthic and pelagic metabolism to the total ecosystem metabolism. We further evaluated the spatial-temporal variation in the metabolic rates (GPP, ER and NEM) along a depth gradient during the growing season (April to October) in the years 2017 and 2018. Our hypothesis was that benthic habitats in a boulder reef area would dominate the whole ecosystem metabolism, especially in the shallow light exposed area. A shift from autotrophic (GPP > ER) to heterotrophic (GPP < ER) state would occur as light decreased with increasing depth. We expect higher ER than GPP in summer, associated with anoxia and release of nutrients from the sediment in deeper areas of the fjord. Finally, elevated oxygen production by macroalgae in the Reef were expected to improve surrounding sediment oxygen conditions compared to the Bare area.

2. Material and methods

2.1. Study area

This study was conducted in the central part of the Limfjorden (Bjørnsholm Bay), an estuary situated in the Northern part of Denmark, connecting the North Sea (salinity of 32–34 psu) in the West and to the Kattegat (salinity of 19–25 psu) in the East (Fig. 1). It is a shallow estuary (mean depth 5.5 m), and covers a total area of 1575 km^2 making it the
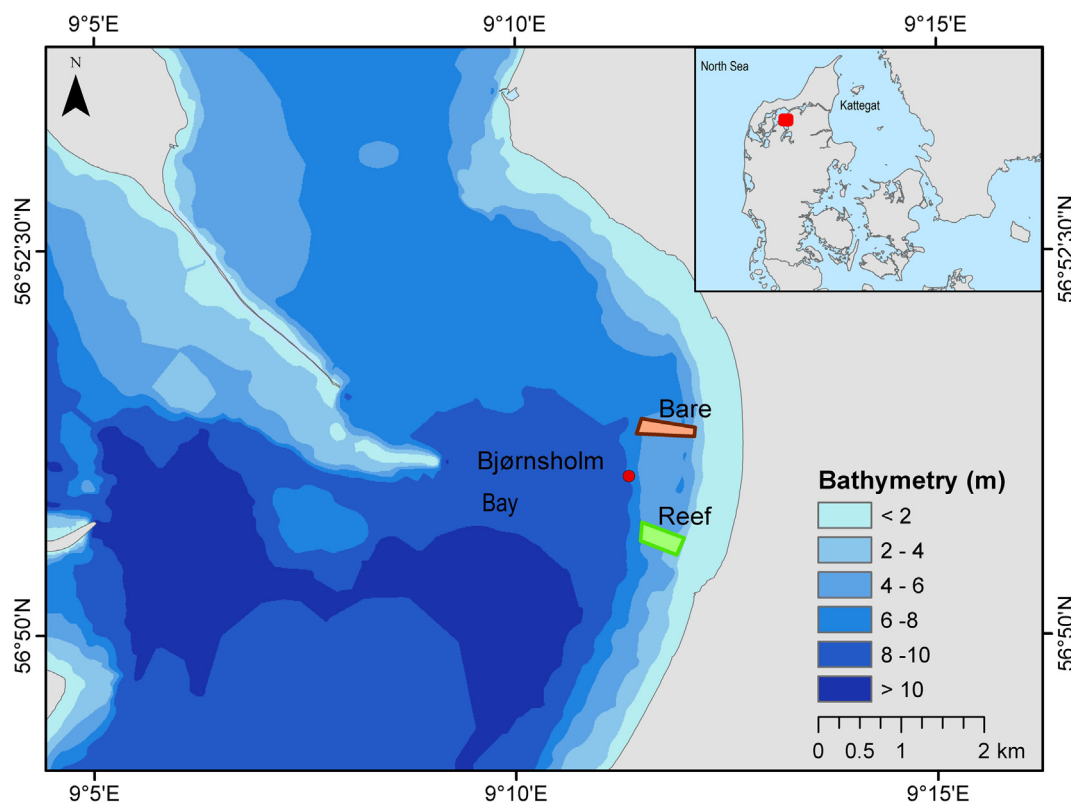
Fig. 1. Map of the Limfjorden situated in the northern part of Denmark. Investigations were performed from 2017 to 2018 in a Reef and a Bare area in Bjørnsholm Bay. The red circle represents the station where water samples were taken to perform water quality measurements.

largest estuary in Denmark. The Limfjorden consists of a mosaic of bays with different salinities and generally high concentrations of nutrients, originating mostly from diffuse sources on land and a high internal release from sediments during late summer (Riemann et al., 2016). Land use in the watershed is dominated by agriculture (70%), natural areas and forests account for 22%, while urban areas make up to 4%. The Limfjorden has experienced an increase in temperature of more than 2 °C over the last three decades (Hansen and Høgslund, 2021), which stimulate the reoccurring hypoxia/anoxia events in the estuary (Hansen and Rytter, 2018). Most of the seafloor is soft and sandy, and scattered boulders in the coastal zone represent between 5% and 10% of the seafloor, with another 5–40% of the seafloor consisting of biogenic reefs, such as mussel and oyster banks (Helmig et al., 2020).

We compare two areas: one dominated by boulders referred to as 'Reef' hereafter, and the other dominated by soft sediment and smaller stones/gravel, referred to as 'Bare'. The Reef area composed a heterogeneous substrate, with presence of varied sizes of stones (areal coverage by boulders >30%), algae and mussel shells. The Bare area, was on the other hand, characterized by more homogenous substrate, with areal composition >90% consisting of sand, mud and mussel shells. The two areas were located approximately 1 km apart in an area of Bjørnsholm Bay (Fig. 1) with similar hydrodynamics. A series of detailed studies were made in both areas to determine seasonal changes in water quality and metabolic rates in the different compartments (open water = system), benthic and pelagic to determine the importance of reefs for oxygen dynamics in shallow coastal zone of the Limfjorden. The overall study design is shown in Fig. A1 and elaborated in Table A1 and in the following text.

2.2. Water chemistry and physics

Water quality sampling for chlorophyll-a (Chl), total nitrogen (TN), total phosphorus (TP), dissolved inorganic nitrogen (nitrate, nitrite and ammonia) and phosphate was carried out every 2–3 weeks from May 2017 to December 2018, within the Danish National Aquatic Monitoring and Assessment Program (NOVANA). Samples were taken from top (1 m depth) and bottom (approx. 7 m depth) of the water column at the monitoring station (Fig. 1). Sampling and analyses followed Danish standard technical guidelines for all water quality parameters (Fossing et al., 1998; Markager and Fossing, 2014). Water densities, calculated from salinity and water temperature, was used to calculate water column stability using the Brunt–Väisälä buoyancy frequency (N, s^{-1}), and from this the depth of the halocline was derived following Jennings et al. (2012).

2.3. Benthic habitat characteristics

Based on diver's observations, sediment characteristics, composition and abundance of benthic macroalgae and macrofauna was determined in triplicates in an area of 25 m^2 in each depth interval during each of four sampling campaigns (Table S1). *In situ* chambers, used for measurements of benthic oxygen fluxes, were also deployed at 2, 4 and 6 m water depth and after approximately 24 h incubation, sediment and boulders within the chambers were collected and brought to the laboratory for further analysis. Here, fauna and flora were identified to species level to the extent possible. We did not account for the abundance of bacteria nor microalgae living on the surface of boulders. The biomass of each species were estimated after drying and burning the samples at 105° and 450 °C, respectively to calculate the ash free dry weight (AFDW). Species data obtained from the *in situ* chambers were then combined with diver observations to upscale the autotrophic and heterotrophic biomass in the 2, 4 and 6 m water depth intervals. In each campaign and area (Bare and Reef), sediment core samples (only soft sediments) were also taken in triplicates from 2, 4 and 6 m water depth for determination of total nitrogen (TN), total phosphorus (TP), organic matter as loss on ignition (LOI) and iron bound phosphate

(Fe-P), in a depth profile covering the top 10 cm of the sediment. Determination of sediment characteristics and nutrient content were made following Fossing (2018). The sediment oxygen penetration depth were also determined from measurements using 500 µm Unisense® microelectrodes according to Jørgensen et al. (1983).

2.4. Metabolic rates

To characterize the ecosystem integrated metabolic rates, we performed continuous open water diel O_2 measurements supplemented with monthly bottle incubation experiments to determine pelagic rates and their contribution. In addition, the *in situ* benthic chamber incubations together with laboratory incubations of collected sediment cores were carried out to determine the benthic rates and their contribution.

2.4.1. Ecosystem metabolism

High frequency measurements of dissolved oxygen, water temperature (miniDOT oxygen optode loggers, PME®), salinity (HOBO U24-002-C ONSET®) and underwater light (HOBO UA-002-64 pendant, ONSET®) measurements were performed from April 2017 to September 2017 in Bare and Reef areas. Light measurements by HOBO sensors were converted from Lux to PAR units using measured conversation factors. In 2018, the loggers were deployed during the same season but only in the Reef area as results (shown later) from 2017 indicated no overall differences in ecosystem metabolic rates between the Bare and Reef area. In each area, loggers were placed near the top (1 m below the surface), and near the bottom (1 m above the bottom depth) of the water column, recording every 30 min (Table S1). Logger sensors were wrapped with cobber netting and cleaned on a monthly basis to reduce fouling and sensor drift. Logger data were screened for outliers and sensor drift was corrected by assuming a linear decline in sensitivity over each month period.

2.4.2. Pelagic metabolism

Monthly pelagic water samples were collected as part of the national Danish monitoring program (NOVANA) at a water quality station in Bjørnsholm Bay (Fig. 1) that represented both the Reef area and the Bare area. We assumed that pelagic metabolic rates were similar in the two areas, located *ca.* 1 km apart. Triplicate water samples were incubated each in 0.9 L transparent glass jars (Staehr et al., 2018) all placed at 1 m depth, and measured at monthly frequency from April 2017 to September 2018. Each bottle was equipped with miniDOT® oxygen optode and temperature logger, and an underwater light logger (HOBO UA-002-64 pendant, ONSET®). Oxygen, water temperature and light were recorded every 10 min, over approximately 24 h for each incubation.

2.4.3. Benthic metabolism

Benthic metabolic rates were determined by two supplementary approaches. The first consisted of *in situ* measurements of oxygen, temperature and light conditions inside large transparent chambers (Staehr et al., 2018), and enabled assessment of contribution from organisms on both soft and hard bottom (boulders). The second determined metabolism from incubation of soft bottom sediment cores in the laboratory. In this way, while *in situ* chambers provided estimates of the oxygen production and consumption from the whole benthic community, including epibenthic fauna, incubations of sediment cores in the laboratory without the epifauna and macroalgae made it possible to estimate oxygen production and consumption from microphytobenthic community and microorganisms.

Benthic chambers were deployed at 2, 4 and 6 m water depth to cover a depth gradient in both the Bare and the Reef area. Triplicate chambers were incubated at each depth and area during four field campaigns (August 2017, October 2017, April 2018 and August 2018), providing a total of 72 *in situ* chamber incubations. Chambers consisted of gas impermeable transparent plastic bags attached to a hard metal collar (*ca.* 30 cm in diameter and 40 cm in height), which was secured firmly into the sediment with metal plugs. Within each chamber, dissolved oxygen and water temperature with miniDOT oxygen optode loggers (PME®), and underwater light (HOBO UA-002-64 pendant, ONSET®) were recorded every 10 min, over a period of approximately 24 h. The volume of each chamber was determined from dilution of a known concentration of color (food dye).

Measurements of oxygen fluxes on sediment cores collected during the four campaigns (Table S1) were made in the laboratory under *in situ* temperature and light conditions. At each of the six stations, divers sampled sediment in Plexiglas core liners (inner diameter 5.2 cm) in replicates of five. The sediment was brought undisturbed to the laboratory, where the cores were pre-incubated for 12 h at *in situ* conditions using bottom water from the location according to Fossing (2018). The oxygen flux in light or darkness, respectively, was expressed in µmol m^{-2} h^{-1} and calculated from oxygen concentration at the start and end of the incubation, the water column height, core diameter and the duration of the incubation period. Negative fluxes specifies an oxygen uptake and positive values a flux from the sediment to the water column. GPP by microphytobenthos was estimated from the O_2 flux during the light and dark incubations, respectively. During dark incubation no O_2 was produced and the rate (FO$_2$ $_{dark}$) expressed as µmol O_2 m^{-2} h^{-1} therefore corresponded to the gross O_2 uptake (*i.e.* FO$_2$ $_{dark}$ ≤ 0). The O_2 flux in light (FO$_2$ $_{light}$) equaled the sum of O_2 gross production and respiration. Thus, GPP = FO$_2$ $_{light}$ − FO$_2$ $_{dark}$. Microphytobenthic GPP values were then multiplied by number of daylight hours to obtain a diurnal GPP expressed in mmol m^{-2} d^{-1} as described by Dalsgaard (2003). Diurnal sediment oxygen uptake, or sediment ER was calculated as hourly sediment oxygen uptake measured in dark multiplied by 24.

2.5. Calculations of metabolic rates and their relative contributions

GPP, ER and NEM were estimated using an inverse modelling approach (Brighenti et al., 2015), where high frequency open water measurements were used to estimate whole ecosystem metabolic rates, bottle incubations for estimating pelagic rates, and chamber incubations for benthic rates. The metabolic rates were estimated according to the Eq. (1):

$$DO_{t+1} = DO_t + GPP_t - R_t + F_t \tag{1}$$

where DO_{t+1} and DO_t are the DO concentrations (mg L^{-1}) at discrete times $t + 1$ and t within 30 min resolution; GPP_t is GPP at time t, R_t is the respiration rate at time t, and F_t is the net exchange of O_2 between the estuary and atmosphere at time t (Eq. (3)). For pelagic and benthic estimates, no air-water gas exchange is expected, thus Eq. (1) was simplified by removing the F_t term. NEM was calculated at hourly (hr) time steps using a light- and temperature dependent model (Eq. (2) following Brighenti et al. (2015)):

$$\Delta DO_{t+1} = NEM_{hr} = P_{max} \tanh\left(\frac{\alpha \times I_t}{P_{max}}\right) - R_{max} \times 1.07^{(T_w - 20)} \tag{2}$$

where P_{max} is the maximum photosynthesis at saturating light levels, α is the initial linear slope of the photosynthesis *vs.* light curve describing the average rate of photosynthesis per unit PAR, and I_t is the surface PAR (µmol photons m^{-2} s^{-1}) measured at time t. Respiration was a function of the rate of ER at 20 °C water temperature (T_w) and a temperature sensitivity constant equal to 1.07. Net oxygen exchange between air and water was calculated according to Staehr et al. (2010), but only for open water measurements:

$$F_t = \left[k_t \left(DO_t - DO_{sat(t)}\right)/Z\right]/2 \tag{3}$$

where DO_t is the measured concentration in the water and $DO_{sat[t]}$ is the concentration in the water at equilibrium with the atmosphere at ambient temperature and pressure (Weiss, 1970) at time t, k_t is the coefficient of gas exchange for DO at time t, and Z is the total depth (in meters) of the water column at the measurement site. The value of k was calculated based on the relationship among Schmidt numbers (Sc) and temperature (Jahne et al., 1987): $k = k_{600} \times (Sc / 600)^{-0.5}$, where $k600$ is the piston velocity calculated as function of wind speed at 10 m height. Hourly wind speed and surface PAR data representing Bjørnsholm Bay were extracted from a meteorological model (WRF) which integrates local measurements (Skamarock et al., 2005).

The three parameters in Eq. (2) (P_{max}, α and R_{max}) were estimated using a conjugate-gradient optimization algorithm that minimized the sum of squared errors between estimated and observed DO for each incubation period with constraints on the parameter space, *i.e.* lower and upper bound for each parameter. The parameters P_{max}, α, and R_{max} were finally used to estimate DO concentrations at every 30 min using Eq. (1) above. For pelagic and benthic estimates, the P_{max}, α and R_{max} parameters were estimated based on seven days of light and temperature measurements, obtained from a nearby meteorological station. From this, we were able to estimate benthic and pelagic GPP, ER and NEM for seven days in each campaign.

To assess how well the model fitted the observed DO data performance, we determined the coefficient of determination (r^2). To reduce bias by erroneously modelled DO curves, we removed sonde days with poor fit ($r^2 < 0.6$). The optimized parameters P_{max}, α and R_{max} were subsequently used to calculate hourly rates of GPP, ER and NEM, inserting 30 min interval recordings of the mean available light in the water column and water temperature over a 24 h period. Mean light availability (E_{mean}) was calculated according to Staehr and Sand-Jensen (2007):

$$E_{mean} = E_0 (1 - e^{-K_D Z}) / K_D Z \qquad (4)$$

where K_D is the diffuse light attenuation coefficient (m^{-1}), Z is the total depth of the water column, and E_0 is the surface PAR ($\mu mol\ photon\ m^{-2}\ s^{-1}$). Daily light attenuation was determined as the slope of a linear regression model of irradiance (E_z) *versus* depth (z) as $\ln(E_z) = b + K_D z$ from light measurements in the water column (Table 1). Daily rates of GPP, ER and NEM were calculated as the average hourly rates (over 24 h) multiplied by 24 h. Volumetric daily rates were then converted to areal-specific rates, considering water depths of 2, 4 and 6 m. For open-water measurements, we averaged volume rates determined at top and bottom, as the water column was well mixed and only small differences in the measured oxygen concentrations between the top and bottom at the individual sites were observed. Subsequently areal rates were calculated by multiplying the volumetric rates with the total depth

Table 1
Autotrophic and heterotrophic biomass and sediment conditions during four campaigns in the Bare and Reef areas in Bjørnsholm Bay. Values are averages ±1 SD of four sampling campaigns (sediment Chl only one campaign) and 3 water depths (2, 4 and 6 m).

Type	Parameter	Unit	Bare area	Reef area
Biomass	Autotrophic	g AFDW m^{-2}	0.07 ± 0.22	15.98 ± 19.10
	Heterotrophic	g AFDW m^{-2}	2.25 ± 4.33	20.49 ± 15.80
	A:H ratio	No unit	0.03 ± 0.05	0.78 ± 1.21
	Sediment Chl	mg m^{-2}	26.8 ± 13.1	22.2 ± 10.2
Composition	Stones > 10 cm	%	0.8 ± 2.9	15.5 ± 12.2
	Stones < 10 cm	%	14.6 ± 30.9	32.4 ± 23.0
	Sand/gravel	%	34.0 ± 48.5	34.8 ± 36.2
	Mud	%	49.5 ± 49.0	11.1 ± 19.6
	Dead shells	%	1.1 ± 2.8	6.6 ± 5.1
Chemistry	LOI	mg g^{-1} DW	22.8 ± 22.6	13.6 ± 7.6
	TN	mg g^{-1} DW	0.98 ± 0.88	0.39 ± 0.30
	TP	mg g^{-1} DW	0.26 ± 0.17	0.20 ± 0.01
	Fe-P	mg g^{-1} DW	0.015 ± 0.028	0.009 ± 0.008
	O_2 pen. depth	mm	1.09 ± 0.68	1.41 ± 0.66

at each site, which gave a final areal unit in mmol $O_2\ m^{-2}\ d^{-1}$. Open water measurements at shallow sites represent 2 m, deep sites represent 6 m and the average of shallow and deep were used to represent rates at the intermediate 4 m depth interval. For bottle incubations, this simply involved multiplying volumetric rates (mmol $O_2\ m^{-3}\ d^{-1}$) with the depth (m) of the water column at the measurement site, resulting areal rates in mmol $O_2\ m^{-2}\ d^{-1}$. For benthic rates, this conversion involved multiplying the rates by the chambers volume (on average 24 L) and dividing by the area of the incubation chambers (0.14 m^2). We present all ER rates as negative values, as it represents oxygen consumption and to facilitate interpretation and rate comparison.

To assess the benthic and pelagic contribution to the total metabolic rates, we estimated the growing season (April to October) total metabolism (GPP, ER and NEM) for each area (Reef and Bare in 2017, and Reef in 2018), and water depths (2, 4 and 6 m depth), and compared with the respective monthly pelagic and benthic rates. For months where benthic rates were not measured (9 out of 13 months), we calculated these as the difference between total and pelagic rates. Comparing measured benthic rates with those estimated as the difference between total and pelagic rates showed a reasonably good agreement, with rates at the similar level, and correlation values between 0.47 (GPP) and 0.67 (ER). For the growing season in 2018, the pelagic rates were initially higher than the total rates, due to high chlorophyll-a concentrations in the surface waters. Therefore, we adjusted the pelagic rates according to the ratio of chlorophyll-a concentration in the surface layer to the average concentration in the whole water column. In doing so, the pelagic rates became comparable to the whole system rates in 2018. Finally, we calculated an overall average of metabolic rates for each area (Reef and Bare in 2017, and Reef in 2018) and water depth (2, 4 and 6 m), considering all sampling periods for total, benthic and pelagic GPP, ER and NEM.

2.6. Statistical analysis

Total metabolic rates (GPP, ER and NEM) from open water measurements were compared among areas (Bare and Reef in 2017, and Reef in 2018) in between three different water depths (2, 4 and 6 m) and months (April–September) using a general linear mixed model (GLMM), using the statistical package *nlme* (version 3.1.140) following Pinheiro et al. (2018). To describe spatial and seasonal variation of metabolic rates, water chemistry and biomass distribution, areas, depths and months were considered fixed factors, and variation among sampling days within months was considered a random factor. For benthic metabolic rates (both *in situ* and lab incubations) and sediment characteristics, a GLMM was applied describing spatial variation among the water depths (2, 4 and 6 m) and areas (Bare and Reef), and temporal variation among the four campaigns as fixed factors, and variation among sampling days within campaigns as random factor. For pelagic metabolic rates, a GLMM was applied describing spatial variation among the depths and temporal variation among the two years (at a monthly resolution) as fixed factors, and variation among sampling days within months as random factor. All statistical analyses were performed using R Studio (version 3.1.140) (R Core Team, 2019). Meteorological (air temperature, wind speed and PAR) and water chemistry parameters were also analyzed using GLMM, comparing the two growing seasons (2017, 2018) and water depths (at a daily/weekly resolution) as fixed factor, and variation among sampling days within years/depths as random factor.

3. Results

3.1. Water quality and oxygen conditions in the water column

The bi-weekly ship based NOVANA sampling revealed a clear seasonality in most physical, chemical and biological conditions in the

water column and for several parameters (temperature, salinity, oxygen, nutrients) a clear distinction between the two studied years (Fig. 2). The growing season of 2018 was warmer, with recorded daily maximum water temperatures (high frequency loggers) of 28.1 °C compared to 22.8 °C in 2017. Meteorological data obtained from the WRF model similarly revealed higher light levels with PAR on average 355 μmol photons m^{-2} d^{-1} in 2018 compared to 326 μmol photons m^{-2} d^{-1} in 2017. These values correspond to 16 and 12 μmol photons m^{-2} d^{-1} at 4 m water depth in 2018 and 2017, respectively. Bottom waters were often slightly (0.5 psu) more saline, and except for short periods with heat spells, during 2018, temperatures were similar throughout the water column (Fig. 2A). The combined salinity and temperature conditions showed a generally well-mixed water column for the upper ca 6 m (Fig. 2C). However, during prolonged periods of warm conditions in August 2018, stratification at around 5 m depth continued for up to 4 weeks, causing oxygen depletion (Fig. 2D) and release of phosphate (Fig. 2F) in bottom waters. A longer period of lowered salinity during winter-spring in 2018 (Fig. 2A) were caused by high precipitation and associated run-off from land (not shown). This was furthermore seen as elevated levels of TN during February–June (Fig. 2E), which during the following warm and sunny summer, stimulated growth of phytoplankton seen as high Chl levels and turbidity (K$_D$) during June–July

2018 (Fig. 2B). Concentration of dissolved oxygen and partial pressure of CO$_2$ in surface waters showed clear opposing seasonal patterns, both indicating dominance of primary production during summer, and respiration during autumn and winter (Fig. 2D). This seasonality was furthermore seen as depletion of inorganic nitrogen during spring and early summer (Fig. 2F).

3.2. Sediment characteristics

The Bare area and the Reef area differed significantly in most sediment characteristics (Tables 1 and A2). The Bare area had low biomass of both auto- and heterotrophs (macroalgae and macrofauna), with sediments dominated by mud, sand/gravel and small stones. Species richness and overall biomass was much higher in the Reef area, with higher levels in the growing season of 2017 compared to 2018 (Fig. A2). In the Reef area, autotrophic species were dominated by macroalgae (*Sargassum muticum*, *Halidrys siliquosa*, filamentous and encrusting algae) and benthic microalgae, with heterotrophic species dominated by sea sponges, sea urchins, ascidians, starfish, crabs, lobsters, snails, small crustaceans (*e.g.* balanus) and polychaetes. In the Bare area, benthic microalgae and encrusting algae were present together with heterotrophs dominated by sea urchins, ascidians, starfish,
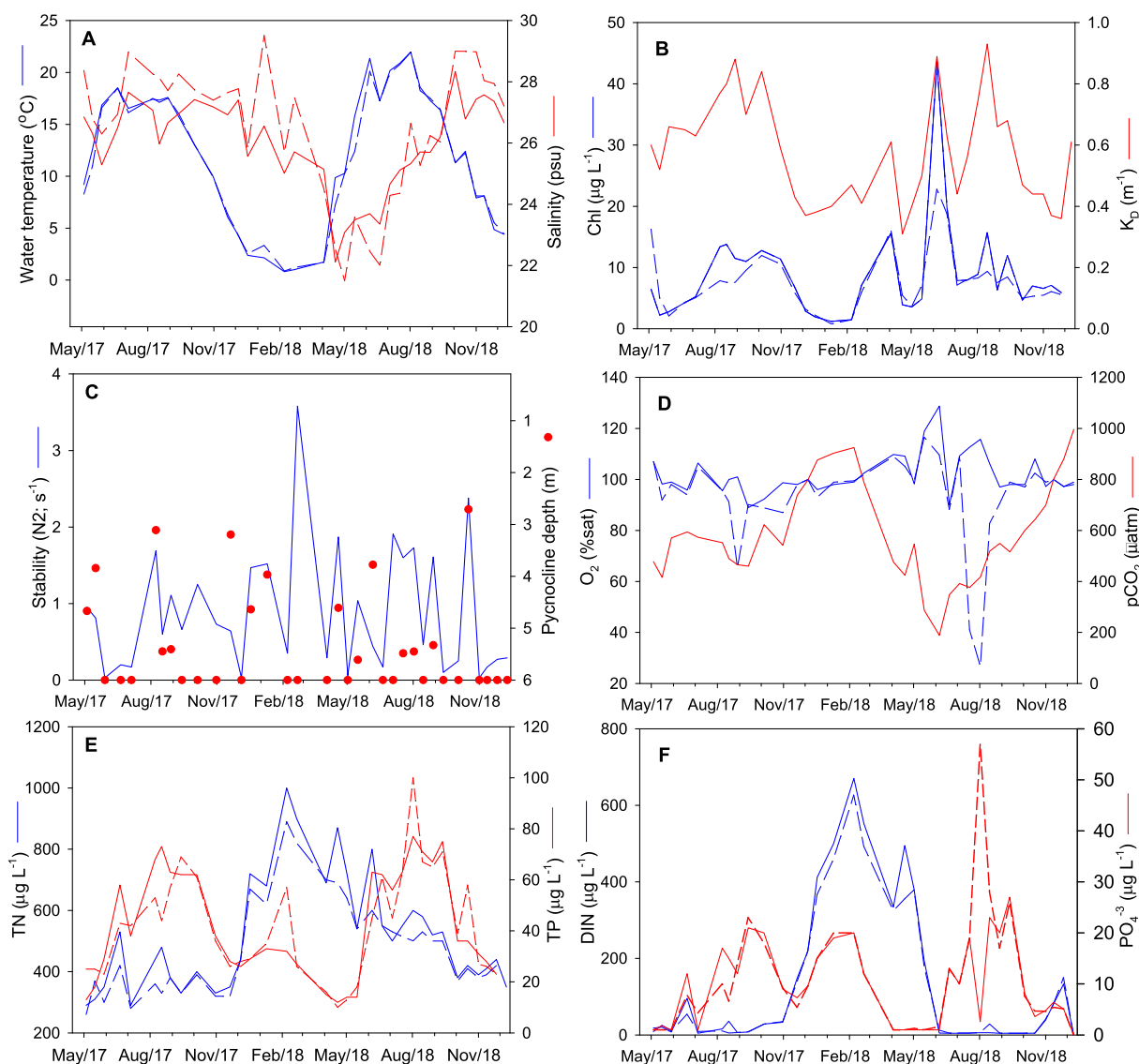

Fig. 2. Water quality, chemical and physical parameters measured during May 2017 to December 2018 in Bjørnsholm Bay, at the top (1 m – full line) and the bottom (6 m – dashed line) of the water column.

polychaetes and small crustaceans. The Bare area sediments were sandy with relative low content of organic matter (LOI) and nutrients (TN, TP and Fe-P). In comparison, the Reef area had several fold higher biomass of both auto- and heterotrophs, was dominated by stones, boulders and sand/gravel, with a slightly lower concentration of both organic matter (LOI) and nutrients (TN, TP), allowing oxygen to penetrate slightly deeper (Table 1). Benthic chlorophyll-a concentrations were relatively high on the collected soft bottom surfaces in both areas, ranging between 6 and 46 mg Chl m^{-2} with similar levels in both areas (Table 1, $p = 0.37$), and an overall tendency for higher values in the deeper waters ($R_{pearson} = 0.50$, $p = 0.02$). Also, based on divers observations, the benthic diatom cover was high, covering approximately 70% of the soft sediments, particularly visible in the shallow Bare area covering >90% of the sand/gravel (Fig. A3).

3.3. Metabolic rates

3.3.1. Ecosystem metabolism

Continuous oxygen and temperature measurements (Fig. A4) in the Reef and Bare area provided more detail to the summer seasonality observed from discrete ship sampling in 2017 and 2018 and confirmed these observations (Fig. 2A, D). Differences between near surface (top) and near bottom (bottom) temperatures were generally below 0.5 °C and not significantly different (Table A2). Also salinity measurements (not shown) did not indicate vertical differences. This indicates that for both growing seasons and in both areas the water column was well mixed. Water temperatures (both bottom and surface) were however, significantly higher in 2018 compared to 2017 ($p < 0.05$, Mann U test), and in 2018 bottom oxygen levels were significantly lower than surface levels (p < 0.05, Mann U test). During the summer season (June to August), bottom oxygen concentrations fell below 4 mg L^{-1} for five days during 2017 compared to 14 days during the much warmer 2018. Overall, temperature and oxygen measurements showed that the water column in both areas were well-mixed. Lower bottom oxygen concentrations in 2018 however, indicated that oxygen consumption in the sediment was stimulated by the higher temperatures.

Rates of open water ecosystem metabolism showed similar seasonal trends and day-to-day variability for the Bare area and the Reef area during 2017 (Fig. 3). There was a significant seasonality effect for GPP and ER in both areas (Table A2, p < 0–0001), with the higher ER than GPP during spring resulting in negative NEM, shifting to a more net autotrophic condition in summer (GPP > ER; NEM > 0; Fig. 3). Metabolic rates in the Reef area showed similar patterns during spring (April and May) in both years. However, after an initial peak in GPP during late May 2018 that lead to net autotrophic (NEM > 0) conditions, the Reef area turned net heterotrophic (NEM < 0) for the remaining part of the season opposite to 2017 (NEM > 0). Comparing seasonal means, the open water overall seasonal NEM was positive in both the Reef and the Bare area in 2017 (NEM = 58 and 48 mmol O$_2$ m^{-2} d^{-1}) compared to negative NEM (-71 O$_2$ m^{-2} d^{-1}) in the Reef area in 2018. Open water metabolic rates were strongly correlated with water temperatures (Table A3). Whereas NEM was positively correlated to water temperatures in both the Reef and the Bare area during 2017, the correlation was negative in 2018. This occurred as ER was more strongly related to increasing temperature than GPP, indicating that ER in particular but also the overall metabolic balance of the system, was significantly influenced by water temperature (Table A3). In addition, NEM values were more strongly correlated with wind speeds in 2018 (Table A3), suggesting a negative effect of resuspension of organic matter settled at the sea floor. Applying a geometric mean regression model of GPP as a function of ER, ER was found to be more strongly coupled to GPP in the Reef area (R^2 = 0.35 and 0.25 in 2017 and 2018 respectively) than in the Bare area (R^2 = 0.05 in 2017). Comparing the 95% CL we found that the slope of ER vs. GPP was not significantly different from 1 in either the Bare area or the Reef area in 2017, while the slope (1.23) was significantly>1 in the Reef area in 2018, providing
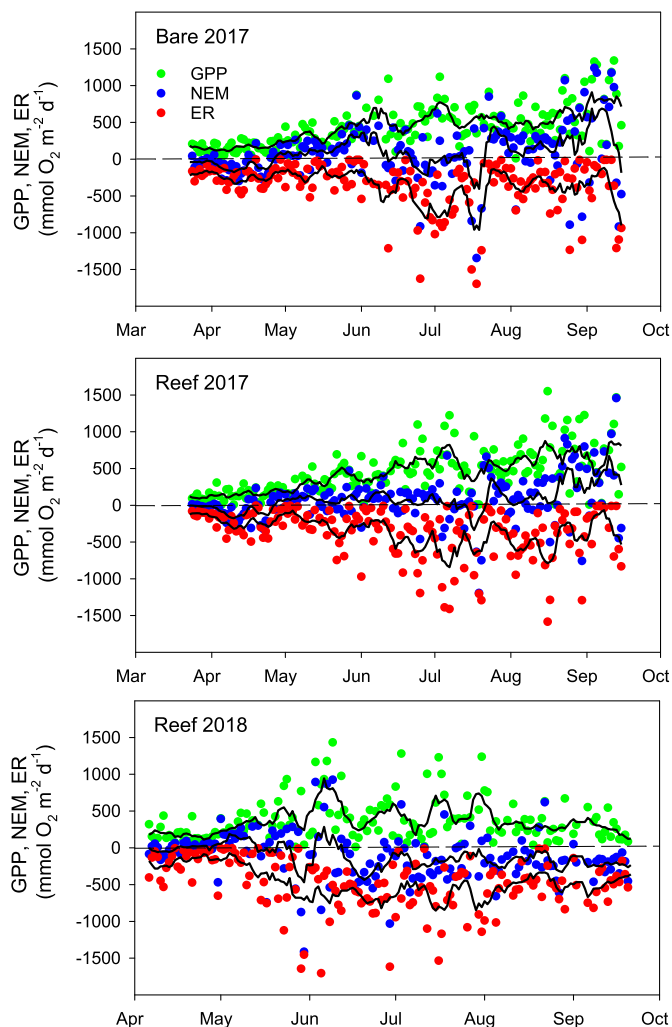

Fig. 3. Daily rates of open water rates of gross primary production (GPP), net ecosystem metabolism (NEM) and ecosystem respiration (ER) for the Bare and Reef areas in 2017, and for Reef in 2018. All rates represent areal rates for a water column of 6 m depth. Solid lines are 7 days running averages.

further support of net heterotrophy here. Also, the intercept of the ER vs. GPP plots was higher in 2018, indicating that background respiration levels were elevated compared to 2017.

3.3.2. Pelagic metabolism

Pelagic rates were only measured at one station in Bjørnsholm Bay (Fig. 1), and assumed to be similar for both the Bare and the Reef area. A significant seasonal variation with higher GPP, ER and NEM was observed during summer months as compared to spring (Table A2, $p < 0.0001$; Fig. 4). Furthermore, GPP and ER were higher in 2018 than 2017. Pelagic NEM was positive except during periods in April and September 2018, indicating a net production of organic material in the water column.

3.3.3. Benthic metabolism

Despite the distinct differences in the biomass and sediment characteristics between the two areas, levels of benthic metabolic rates were similar, with higher metabolic rates in the summer and lower rates in spring and autumn, and a general net heterotrophic condition (NEM < 0; Fig. 5). In general, benthic GPP rates were higher at shallow waters (2 m) decreasing with depth, while ER was highest at 4 m depth. Despite low autotrophic biomass, higher GPP rates were found in the Bare area, suggesting a significant contribution from microphytobenthic primary production. In situ chamber and lab measurements had a

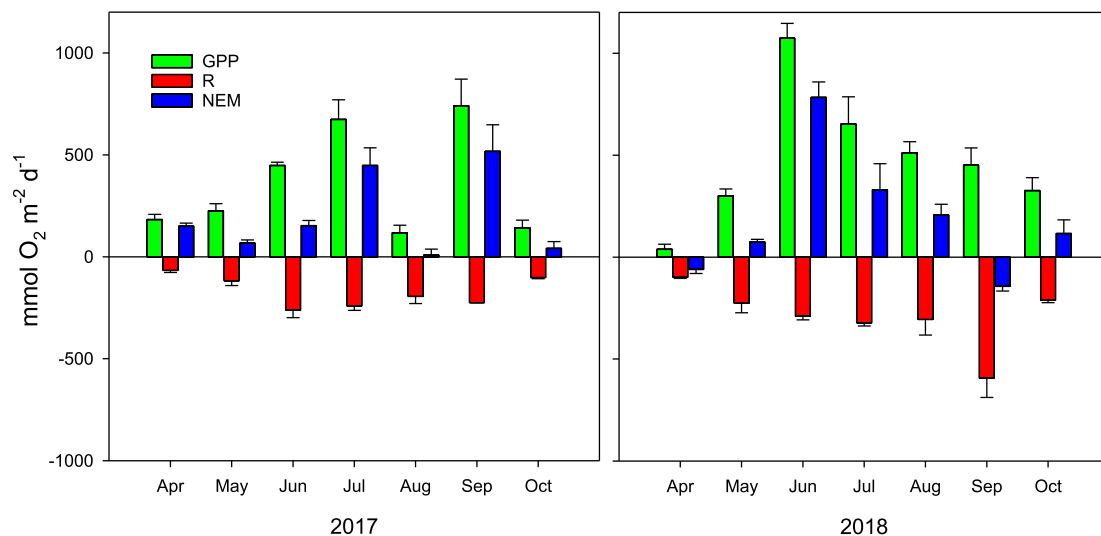**Fig. 4.** Seasonality in pelagic metabolic rates covering monthly estimates from April 2017 to October 2018 at 6 m depth. Error bars indicate the standard deviation from 3 replicates.

similar pattern of variation with depth and among campaigns. Except for August 2017, seasonality in lab measurements was similar to *in situ* measured rates, representing on average 45% of the benthic GPP measured *in situ* in the Bare area and 60% in the Reef area. The same was true for benthic oxygen consumption (ER in lab), which was 49 and 88% of the *in situ* oxygen consumption in the Reef and Bare area respectively, further indicating a high importance of the microphytobenthic communities. There was no significant difference in microphytobenthic gross primary production (MPP) between the two areas ($p > 0.05$), but as expected, MPP decreased as the water depth increased ($p < 0.05$). Also, both *in situ* and in lab rates of GPP and ER varied significantly between months (Table A2, $p < 0.0001$), suggesting seasonality effect for all measured benthic rates. For ER measured in lab, higher ER was observed in the Bare area ($p < 0.05$), with an increase with depth ($p < 0.05$), and the highest ER in August 2018 ($p < 0.0001$). Relating *in situ* benthic respiration rates with the total biomass (autotrophic plus heterotrophic biomass) of the chambers showed a moderately positive relationship ($R_{pearson} = 0.36$, $p = 0.001$).

3.3.4. Pelagic and benthic contribution to the whole ecosystem metabolism

Monthly estimates of the metabolic contributions from total, pelagic and benthic metabolic habitats to GPP, ER and NEM are shown in Figs. A5 and A6 and summarized for the growing seasons in Figs. 6 and 7. Overall, we found that ecosystem (total) GPP and ER increased with depth regardless of the areas and growing season (year), with benthic rates accounting for 47% of total GPP for shallow (2 m) and 2% in deeper (6 m) waters. Similar results were found for total ER as benthic processes represented overall 53% (2 m) and 6% (6 m) of the total ER. In comparison, the benthic ER was at similar levels across depths. As a result, the benthic compartment became increasingly net heterotrophic with depth in both the Reef and the Bare area. Although respiration dominated the benthic compartment, with increasingly negative NEM with depth, the water column (total) NEM values remained positive during the 2017 season in both areas as pelagic GPP ≫ pelagic ER. While pelagic GPP > ER in 2018, the much elevated benthic respiration in 2018 resulted in an overall net heterotrophic state (NEM total < 0) for the Reef area in 2018 (Figs. 6, 7 and A6).

4. Discussion

This study investigated the importance of a boulder reef for oxygen dynamics, water quality and sediment conditions in the Limfjorden, through a direct comparison of the importance and trends of benthic and pelagic processes along a gradient from 2 to 6 m water depth in

the years 2017 and 2018. During an intensive sampling of two growing seasons (April to October), we quantified oxygen dynamics and water quality conditions, sediment characteristics and benthic species abundance in an area characterized as a boulder reef (Reef) compared to a nearby bare soft bottom area (Bare). Overall, only minor differences were found in the oxygen dynamics between the Reef and Bare area, despite clear differences in substrate conditions and coverage of flora and fauna. This is mostly explained by very high pelagic rates, a low macroalgae coverage, low benthic light availability, and high dominance of heterotrophs in the Reef area. We furthermore documented significant productivity by the benthic microalgae in both areas, and a shift from autotrophic in 2017 to heterotrophic conditions in 2018 caused by higher benthic respiration stimulated by higher temperatures and organically enriched sediments.

4.1. Oxygen dynamics around a boulder reef

Continuous open water oxygen measurements and derived ecosystem metabolism rates showed large variations in ecosystem level GPP, ER and NEM on a daily basis and across seasons and habitats. The magnitude and temporal variability in these open-water metabolic rates resembled those found in the North American estuaries (Caffrey, 2004) and in the Danish Roskilde Fjord (Staehr et al., 2018). The seasonal average GPP was slightly higher in this study in Bjørnsholm Bay (range 7.6–9.4 g O_2 m^{-2} d^{-1}) compared to similar measurements in Roskilde Fjord (range 6.0–6.5 g O_2 m^{-2} d^{-1}), but similar to average values reported by Hoellein et al. (2013) for estuaries across the Northern Hemisphere (~10 mg O_2 m^{-2} d^{-1}). In comparison, seasonal average ER ranged 7.2–9.9 g O_2 m^{-2} d^{-1} compared to 5.1–5.9 g O_2 m^{-2} d^{-1} in Roskilde Fjord and 14 mg O_2 m^{-2} d^{-1} for a larger data set (Hoellein et al., 2013), indicating that measurements in this study are within the expected range. There was strong coupling between GPP and ER in the Reef, which had higher plant and animal biomass, consistent with previous studies showing coupling between GPP and ER is largely determined by the habitat type (Caffrey, 2004; Staehr et al., 2018).

Seasonality in total metabolic rates resembled previous studies in temperate waters, with higher productivity and autotrophic conditions during summer and heterotrophic conditions during low light and cooler early spring and autumn periods (Staehr and Sand-Jensen, 2007; Laas et al., 2012; Staehr et al., 2018). Increasing levels of net heterotrophy was, however, observed in the Reef area during summer 2018, mostly driven by elevated rates of benthic respiration. There seems to be several reasons for this shift. Most importantly, the Reef area experienced a loss of autotrophic biomass, and became largely
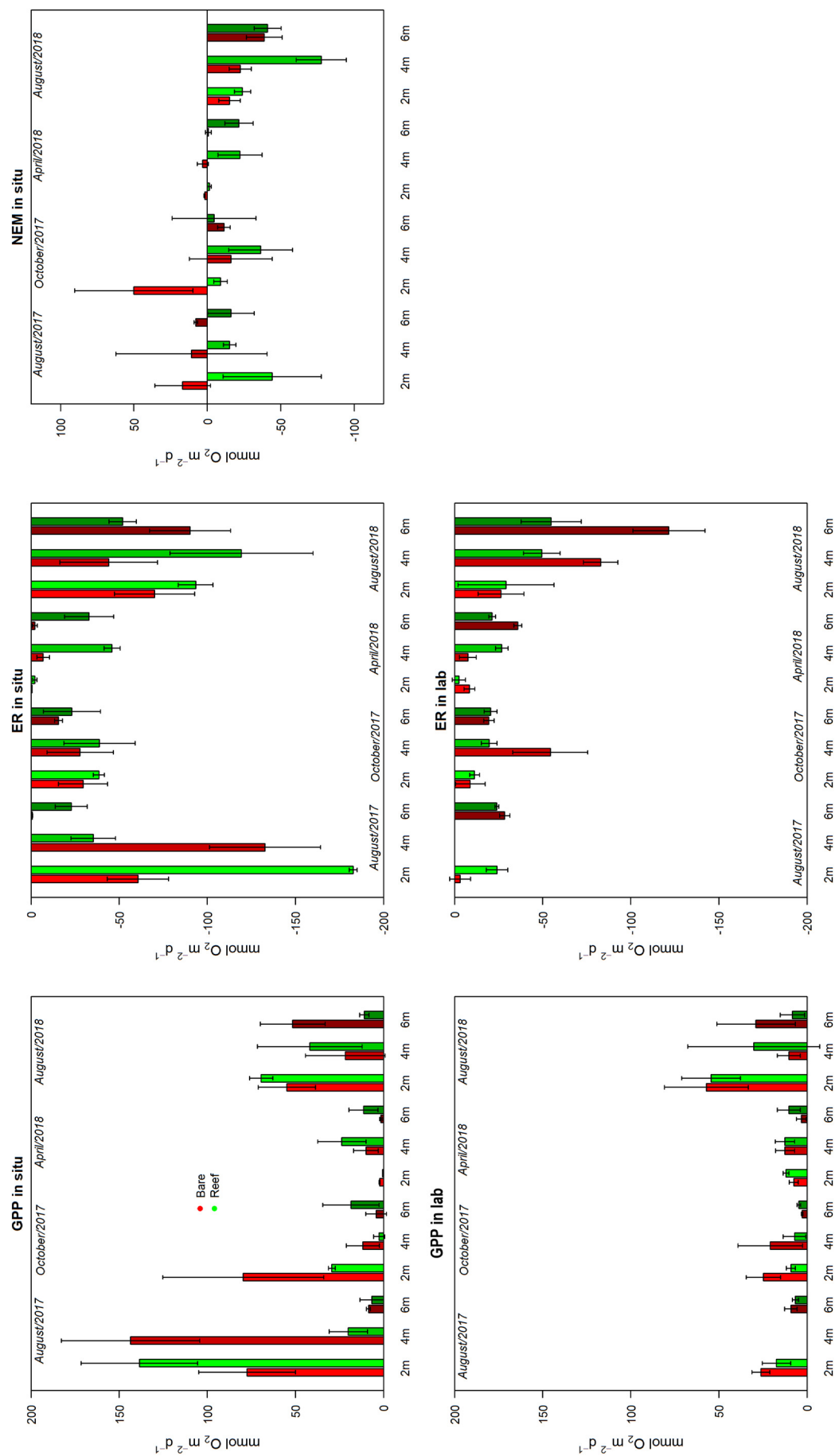
Fig. 5. Seasonality in benthic metabolic rates in 2017 and 2018 for Bare and Reef areas sampled in different depths in Bjørnsholm Bay. Error bars indicate the standard deviation of 3 replicates. Microphytobenthic primary production (MPP) in Bare and Reef areas in the different depths in Bjørnsholm Bay estimated from lab incubations in light and dark. Error bars indicate the standard deviation of 3 to 5 replicates. ER in lab represents the oxygen consumption from the benthic microalgae.
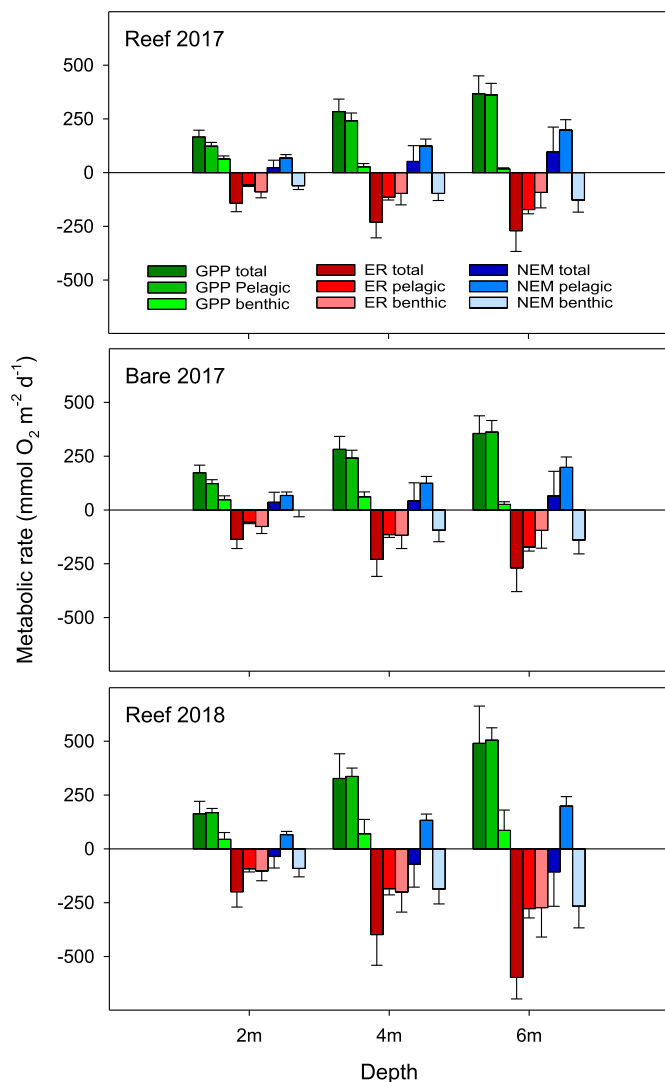
Fig. 6. Comparisons of growing season averages between total, pelagic and benthic rates measured in Bare and Reef areas in 2017 and 2018. Error bars indicate the confidence interval of 3 replicates in each month for pelagic and benthic rates and 2–4 replicates in each month for whole ecosystem metabolic rates.

dominated by oxygen consuming heterotrophs (Fig. A2). Also, a large early summer phytoplankton bloom (Fig. 2B) resulted in high loads of dead and living phytoplankton cells, exopolymers and inorganic constituents clearly visible at the sediment surface (diver's observations) in 2018. Followed by high summer temperatures, this stimulated benthic respiration rates pushing the system towards a heterotrophic state. The importance of high water temperatures as those experienced during summer of 2018 will likely continue to impact the metabolic balance in the Limfjorden, as water temperatures have increased by approximately 2 °C over the last 3 decades (Hansen and Høgslund, 2021) along with a significant increase in the frequency of marine heatwaves in the Limfjorden (Nepper-Davidsen et al., 2019). The high organic matter deposition also made the seafloor very sensitive to resuspension (diver's observation) during summer 2018, reducing underwater light conditions and thereby benthic primary production, further contributing towards heterotrophic state. Moreover, during late summer of 2018, the sediments were highly reduced and covered by a microbial community of the hydrogen sulfide oxidizing bacteria *Beggiatoa* sp. (Fig. A7). This biological mediated sulfide oxidation further added to enhanced oxygen consumption at the seafloor, and microsensor measurements showed that oxygen barely reached the sediment surface in any of the sampled areas. Mobile fauna at even 4 m water depth were observed to be severely affected by the oxygen depletion (Fig. A8). The fact that this occurred even within the shallow Reef area (4 m), show that oxygen production by benthic algae at the Reef was low and incapable of sustaining oxygen for local fauna communities.

4.2. Contribution from benthic and pelagic habitats

Assessment of the relative contribution from benthic and pelagic habitats to total ecosystem integrated rates are rarely performed on the basis of measurements, although attempts have been made in some studies (Oliver and Merrick, 2006; Gawne et al., 2007; Sadro et al., 2011; Staehr et al., 2018). The heterogeneity of benthic habitats, in particular, makes it challenging to investigate their potential contribution to the overall metabolism, and it is therefore often assumed that this can be determined as the residual between open water and pelagic rates (*e.g.* Staehr et al., 2012; Murrell et al., 2018). In this study, we performed 72 *in situ* benthic chamber incubations along depth gradients during the course of 4 sampling campaigns. While the benthic incubation technique is advantageous for comparison of different depth strata, and provides detail of the processes occurring at different depths to evaluate the importance of benthic community, it is still prone to uncertainties from not capturing the true heterogeneity of the extensive
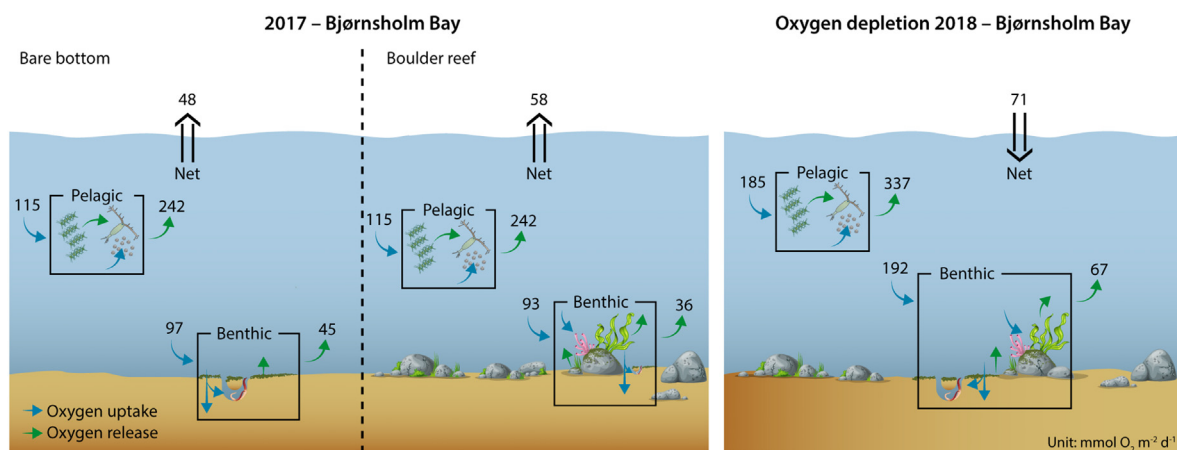

Fig. 7. Syntheses of the results obtained in a depth gradient in the reef area of Bjørnsholm Bay in the Limfjorden during two years (April to October), based on measured processes in total, pelagic and benthic compartments. In 2017, there was a positive net oxygen balance (NEM > 0), which was due to the pelagic GPP > ER. In 2018, the net oxygen balance was negative (NEM < 0). The figure represents rates based on different types of independent measurements, which means that the total budget does not match completely.

benthic habitat. An alternative is the eddy correlation technique, which can discriminate productivity between a seagrass site and a nearby non-vegetated site (Hume et al., 2011). Our direct chamber approach, however, made it possible to obtain replication over several periods and depths, and through comparison with rates obtained from incubation of sediment cores, evaluate the importance of benthic microalgae at the sediment surface.

Similar to studies in Chesapeake Bay (Kemp et al., 1992), and Roskilde Fjord (Staehr et al., 2018), we found that pelagic respiration exceeds benthic oxygen consumption as water column depth increases. In Roskilde Fjord, the benthic habitats contributed up to 90% of total GPP and ER in the shallow vegetated areas, compared to only 40% for GPP and 60% for ER in the shallow vegetated Limfjorden Reef area. The much lower benthic contribution here links to a reduced plant cover, where only less than 20% of the available hard substrate was covered by macroalgae within the Reef area. This indicates that macroalgae growth conditions were either unfavorable due to poor light conditions and/or indicate high loss rates, *e.g.* from grazing. Analysis of long-term trends in macroalgal cover in the Limfjorden has recently documented that the accumulated cover of canopy macroalgae is now only half of what it was in the early 1990s (Stæhr et al., 2019). This development has occurred despite of moderate improvements in underwater light conditions related to reductions in nutrient inputs (Riemann et al., 2016). However, modelling indicates that high densities of sea urchins grazing may severely impact the macroalgal coverage in the Limfjord (Carstensen and Dahl, 2019). This furthermore indicates that a high proportion of benthic primary production is channeled through herbivores, similar to other eutrophic coastal systems (McGlathery et al., 2007). Observations during this study supports the likely negative effect of grazing by the green sea urchin (*Psammechinus miliaris*) as these were very abundant, with densities up to 20 per m^2, and there were clear signs of grazing damage to the canopy of the dominant macroalgae, *Sargassum muticum* (divers observation). Loss of macroalgal coverage through grazing combined with poor light conditions would obviously limit the potential for benthic primary production in the Reef area. However, comparing GPP measured within *in situ* chamber with GPP from laboratory incubations of cores showed a substantial contribution (between 45 and 60%) from microphytobenthos to benthic GPP in both the Reef and Bare area. This helps explain why only minor differences were observed in the benthic contribution to ecosystem (total) GPP between the Reef and Bare area. Nevertheless, the phytobenthic rates estimated in this study were in the lower range of those observed in other benthic habitats (Dalsgaard, 2003).

High nitrogen and phosphorus loads to the Limfjorden have promoted eutrophication leading to enhancement of pelagic primary production at the expense of benthic production by macroalgae and seagrasses (Krause-Jensen et al., 2012). While P-loadings have diminished significantly, reduction in N loadings were much smaller, leading to minimal improvements in water quality and seagrass coverage (Riemann et al., 2016). Anoxia events frequently occur in the deeper parts of the estuary, especially in wet years with large N-inputs from land during winter/spring, stimulating phytoplankton growth during spring/early summer, and decay during warm periods which, typically co-occur with water column stratification (Hansen and Rytter, 2018). Release of N and P from the organically enriched deep sediments, during low oxygen summer conditions, may further stimulate later occurring anoxia events. We observed this following a wet winter and spring in 2018 where increased N concentrations stimulated phytoplankton production reducing water clarity. Deposition of this bloom increased benthic respiration over the summer shifting whole system NEM to net heterotrophy compared to net autotrophy in 2017.

Distinguishing between benthic or pelagic metabolic processes is essential for our understanding of ecosystem functioning (Stutes et al., 2007). In agreement with previous observations for shallow eutrophic temperate estuaries, we documented dominance of pelagic processes (Borum and Sand-Jensen, 1996; Krause-Jensen et al., 2012). Similar to

Meyercordt et al. (1999) we find that light conditions define the relative significance of pelagic and benthic primary production. Under eutrophic conditions, phytoplankton grows at the expense of benthic vegetation, which becomes shaded (Valiela et al., 1997), as reduced light conditions at the seafloor severely limits primary production here (Sand-Jensen and Borum, 1991; McGlathery et al., 2007). Dominance of benthic primary production thus requires clearer water and abundant plant cover at shallow depth (Eyre et al., 2011; Staehr et al., 2018). Benthic microalgae were however, found to sustain high levels of primary production in the bare, non-vegetated area. This supports earlier studies (Stutes et al., 2007; Eyre et al., 2011) of the importance of benthic microalgae for net ecosystem productivity in shallow marine systems. While benthic primary production was low, benthic respiration, strongly contributed to the overall oxygen consumption of the system, especially during warmer and richer nutrient condition periods.

4.3. Reestablishment of boulder reefs in the Limfjorden

Boulder reefs provide a habitat for countless autotrophic and heterotrophic organisms providing essential ecosystems functions, particularly through primary production that serves as the basis for the food chains (Borum and Sand-Jensen, 1996; Gattuso et al., 2006). The stone removal throughout the years in the Limfjorden have changed the sediment conditions towards a more unstable and fine sediment with higher organic matter deposited into the sediments throughout years, thereby limiting the conditions for establishment of macrophytes. The relatively bare seafloor in the Limfjorden makes it very prone to resuspension (Canal-Vergés et al., 2016), with negative effects on water column light availability, and abundance of macroalgae and seagrasses (Riemann et al., 2016; Stæhr et al., 2019) affecting the nutrient and oxygen dynamics of the system. However, the microphytobenthic community is also an important component of the benthic system, especially in shallow areas, where the sediment is within the photic zone (Dalsgaard, 2003). We observed that the Bare area with finer substrate composition, had similar levels of benthic primary production and respiration, as the nearby Reef area having more complex substrate conditions and higher abundance of macroalgae and associated fauna.

The ability of boulder reef communities to contribute towards the improvement in oxygen conditions in Danish waters was originally suggested from ecological modelling results (Møhlenberg et al., 2008). The assumption here was that reefs, due to their abundant cover of perennial macroalgae presumed low light requirements for growth, would accumulate inorganic nutrients from the water column. Through enhanced primary production, they were also expected to significantly oxygenate the surrounding water column and nearby sediments thereby reducing the release of nutrients from the sediment, further reducing phytoplankton growth in the water column and thereby the occurrence of anoxia (Møhlenberg et al., 2008). These expectations were however, not supported by this study, which documented very low contributions from the benthic community to the overall oxygen dynamics of the estuary. This is mostly a result of poor light conditions, reducing both the abundance of macroalgae in the system, and their oxygen production. Recent studies furthermore show that the minimum light requirements for the dominant macroalgae species to produce more oxygen than they respire is up to 6 times higher compared to earlier experimental results (Staehr et al., 2020). There are therefore several reasons why reef areas with current low macroalgal cover and poor light conditions do not improve oxygen conditions and water quality in their local surroundings. Poor underwater light conditions hindered macroalgal growth, thus reducing primary production and their potential significant contribution to the improved oxygen conditions of the system. Moreover, inputs of external nutrients associated with warmer temperatures lead the ecosystem to a more oxygen depleted condition, due to high organic matter decomposition in the sediment and the associated high benthic faunal respiration.

Ongoing mitigation and restoration efforts aimed to improve water quality conditions in the Limfjorden, are expected to gradually raise the importance of benthic processes in the estuary. The pace of recovery is however slow (Riemann et al., 2016) indicating significant lags in ecosystem responses (Duarte et al., 2009) such as improvements in water clarity. Investigations during summer conditions have shown that the minimum light requirements for the dominant macroalgae in the Limfjorden (42–100 µmol photons m^{-2} s^{-1}) (Staehr et al., 2020) are similarly to those of the dominant seagrass *Zostera marina* in the estuary (Staehr and Borum, 2011) for measurements on whole plants. Comparing with light availability shows that a reduction in light attenuation from 0.6 m^{-1} to 0.35 m^{-1} (42% change, summer mean values) is required in the Limfjorden before benthic vegetation is expected to contribute positively to oxygen production within the upper 6–7 m (Staehr et al., 2020). Similar findings were obtained in Carstensen et al. (2013), suggesting that the key to restore widespread and stable populations of eelgrass was to improve light conditions, by reducing nutrients inputs and phytoplankton concentration. As around 73% of the total light attenuation (excluding attenuation by water itself) in the Limfjorden is currently caused by particulate and dissolved organic matter in the water column, recovery of light conditions and benthic vegetation is expected to be a very slow process, delayed by the large accumulated pool of nutrients and organic matter in the system and slow decay of these (Staehr et al., 2020).

Although reversal from a eutrophic state is complex and associated with considerable time lags (McGlathery et al., 2007; Duarte et al., 2009) there are indications that a shift towards benthic dominance through time is, possible. In Roskilde fjord, another strongly disturbed Danish estuary, a gradual transition towards greater contribution and importance of benthic processes (Staehr et al., 2018) in shallow waters was associated with relatively clearer water, providing sufficient light for dense seagrass meadows (Staehr et al., 2017). While pelagic processes earlier contributed between 62 and 100% of total areal GPP in Roskilde Fjord (Borum and Sand-Jensen, 1996), improved water clarity has gradually led to expansion of eelgrass in the system, contributing up to 90% of areal GPP in shallow areas (Staehr et al., 2018), and to a system which gradually has become more net autotrophic (Staehr et al., 2017). Presence of macroalgae, even for a short period, can similarly increase the oxygen production significantly, and shift the system from a heterotrophic to autotrophic state (Dalsgaard, 2003; McGlathery et al., 2007).

5. Conclusions

This work provided a detailed account of the importance of benthic processes to overall ecosystem oxygen dynamics through intensive sampling and measurements of metabolic rates in different habitats. Comparing a vegetated boulder reef with a nearby non-vegetated bare area did not as expected, support expectations of a significant positive impact of boulder reefs to local oxygen dynamics, sediment conditions and water quality. Measurements during two growing seasons showed that under current poor light conditions, in combination with warm and nutrient enriched environment, boulder reefs turn into oxygen graves further enhancing local anoxia events rather than buffering these. High light requirements by perennial macroalgae, dominance by heterotrophic organisms, increasing temperatures and increasing frequency of marine heat wave events in the Limfjorden therefore make boulder reefs very vulnerable to reduced light conditions. A significant improvement in the overall light attenuation is, however, expected to take decades as this requires a significant reduction in the organic matter concentration. Under current turbid conditions, it is therefore not recommended to establish boulder reefs in deeper parts (>3 m) of the estuary, as they will likely become dominated by oxygen consuming fauna. Given that a positive ecosystem effect of boulder reefs requires large nutrient reductions, establishment of reefs cannot be recommended as an N-mitigation tool at the current state of the estuary.

CRediT authorship contribution statement

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.150261.

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