Quantifying clearance rates of restored oyster reefs using modular baskets

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Abstract

Oyster reefs are one of the most threatened marine ecosystems, prompting substantial global efforts to restore them. While biodiversity gains of restored reefs are well documented, other ecosystem services, such as water filtration, remain overlooked. This study tested whether modular baskets could provide a practical way to measure water filtration by bivalve communities on restored oyster reefs and assess community responses to light, a simulated heatwave, and a simulated flood. A seawater system was designed to host ten restoration baskets that had been deployed intertidally for 19 months in Moreton Bay, Australia. We measured baseline clearance rates and then tested the effects of (1) light by covering tanks with or without black polyethylene, (2) temperature by heating half of the tanks ~4 °C above ambient for five days, and (3) reduced salinity by addition of freshwater from ambient (~36) to ~25 or ~15 respectively. *Nannochloropsis oceanica* (CS-246) was added at a density of 1–1.5 × 10^6 cells mL^{-1}, and clearance rates were measured every 30 min for 2 h. Mean baseline clearance rates were 119.06 L h^{-1} ± 14.76 SE. Clearance rates were generally reduced by ~1/3rd when the salinity was ~15, but were not affected by light (light vs dark) or temperature (ambient vs +4 °C). Our results demonstrate modular restoration baskets can be used to better understand the ecosystem services provided by restored reefs and to assess the vulnerability of natural and restored bivalve communities to current and future threats such as heatwaves and floods.

Introduction

Oyster reefs form when living bivalves aggregate on rigid substrates in subtidal and intertidal areas (Kennedy and Sanford, 1999; Beck et al. 2011). These tightly bound molluscs create distinct communities that engineer their surrounding environment (Kasoar et al. 2015). The majority (~85%) of the world's oyster reefs have been lost because of overharvesting, habitat loss, pollution, and disease (reviewed by Beck et al. 2011; Gillies et al. 2018; Gilby 2018). In North America, Europe, and Australia, several oyster reef habitats are functionally extinct (Gillies et al. 2020). Numerous efforts are underway globally to restore oyster reefs using man-made constructions of discarded shells or crushed concrete (e.g., Coen and Luckenbach 2000).

Most studies that have investigated the efficacy of artificial structures in restoring oyster reefs have measured changes in biodiversity (e.g., Gilby et al. 2019; Xu et al. 2023). The capacity of artificial oyster reefs to restore other ecosystem services, such as water filtration, remains unclear. One of the reasons for this is the difficulty of measuring ecosystem services *in situ*. Current methods to measure the amount of water filtered by filter feeders (hereafter clearance rate) are limited in size and volume, are not easily replicated, are impractical for manipulative experiments, and vulnerable to tide and weather (Hansen et al. 2011). Some studies have attempted to overcome these limitations by measuring clearance rates of bivalves in the laboratory (e.g., Castle and Nathan 2022; Cottingham et al. 2023). However, studies on single species in the laboratory often overestimate clearance rates by as much as an order of magnitude compared to communities measured in the field (Hansen et al. 2011). To further refine restoration efforts, a practical method to measure clearance rates of whole communities living on restored oyster reefs is needed.

In this study, we tested the potential for modular oyster reef restoration baskets to be used to measure clearance rates *ex situ* and for manipulative experiments measuring the robustness of bivalve communities living on restoration structures. We used small *ROB 400* reef restoration structures (Fig. 1) to provide baseline data on clearance rates of invertebrate communities colonising restoration structures and then tested how clearance rates responded to variation in light, increased temperature such as during a heatwave, and decreased salinity such as during an extreme flood event.

Methods

Robust Oyster Basket 400

This study used Robust Oyster Baskets 400 (hereafter ROB 400), an artificial oyster reef restoration structure developed by the not-for-profit organisation OzFish Unlimited (Fig. 1a,b). The prism-shaped design (400 × 400 × 300 mm) is made of steel mesh encasing recycled oyster shells (Fig. 1a) which, after deployment, are colonised by mixed communities dominated by filter-feeding bivalves (particularly oysters)(Fig. 1b). Ten ROB 400s were collected (July 2023) after 19 months of deployment in an intertidal oyster lease in Moreton Bay (-27.45684, 153.39528) and housed individually in aerated 110-L polyethylene tanks (Fig. 1c) at the University of Queensland's Moreton Bay Research Station, Minjerribah, Queensland, Australia from July to October 2023. Seawater at ambient temperature (35–37 salinity, ~21 °C) was recirculated among the 10 tanks containing ROB 400s, 6 bare tanks, and a 400-L sump with mechanical filtration (Fig. 1c). ROB 400s were fed a mixed diet of live *Nannochloropsis oceanica* (CS-246, CSIRO Australian National Algae Culture Collection, Hobart, Tasmania, F media, 25 °C, ~35 salinity, 22:2 h light/dark photoperiod) and Shellfish Diet 1800 (Reed Mariculture) fed three times per week. Seawater (50–66 %) was exchanged monthly. Temperature, salinity, oxygen, and ammonia were monitored with a Horiba U-52 Series MultiParameter Water Quality Meter or API Ammonia NH_3/NH_4^+ test kit, respectively. Values are reported in Table S.B.2. ROB 400s were left undisturbed for at least two weeks between experiments.

Baseline clearance rates

To quantify baseline clearance rates, we measured changes in the density of living microalgae (*N. oceanica*) in tanks with ROB 400s compared to tanks without ROB 400s. At the beginning of the experiment, water flow was turned off, which created 10 independent tanks housing ROB 400s and six independent tanks without ROB 400s. Each tank was continuously aerated to maintain dissolved oxygen levels and keep microalgae in suspension. Live *N. oceanica* was added to each tank at an initial mean density of 1,275,667 cells mL^{-1} ± 52,431 SD. Absorbance (sum of λ750, λ664, λ647, λ630) was measured at the beginning of the experiment and every 30 min thereafter until 2 h had elapsed (Hach DR 5000™ UV-Vis). Data on the density of *N. oceanica* in each tank at each time point was generated from absorbance data (supplementary Fig. S.A.1). Clearance rates were then calculated for each replicate per Eq. 1 modified from Riisgård (2001):

$$Cl = (V/t)\, ln(C_0/C_t) \tag{1}$$

where C_0 and C_t equal the concentration of microalgae (cells mL^{-1}) at the time points zero and t respectively, and V equals the volume of water.

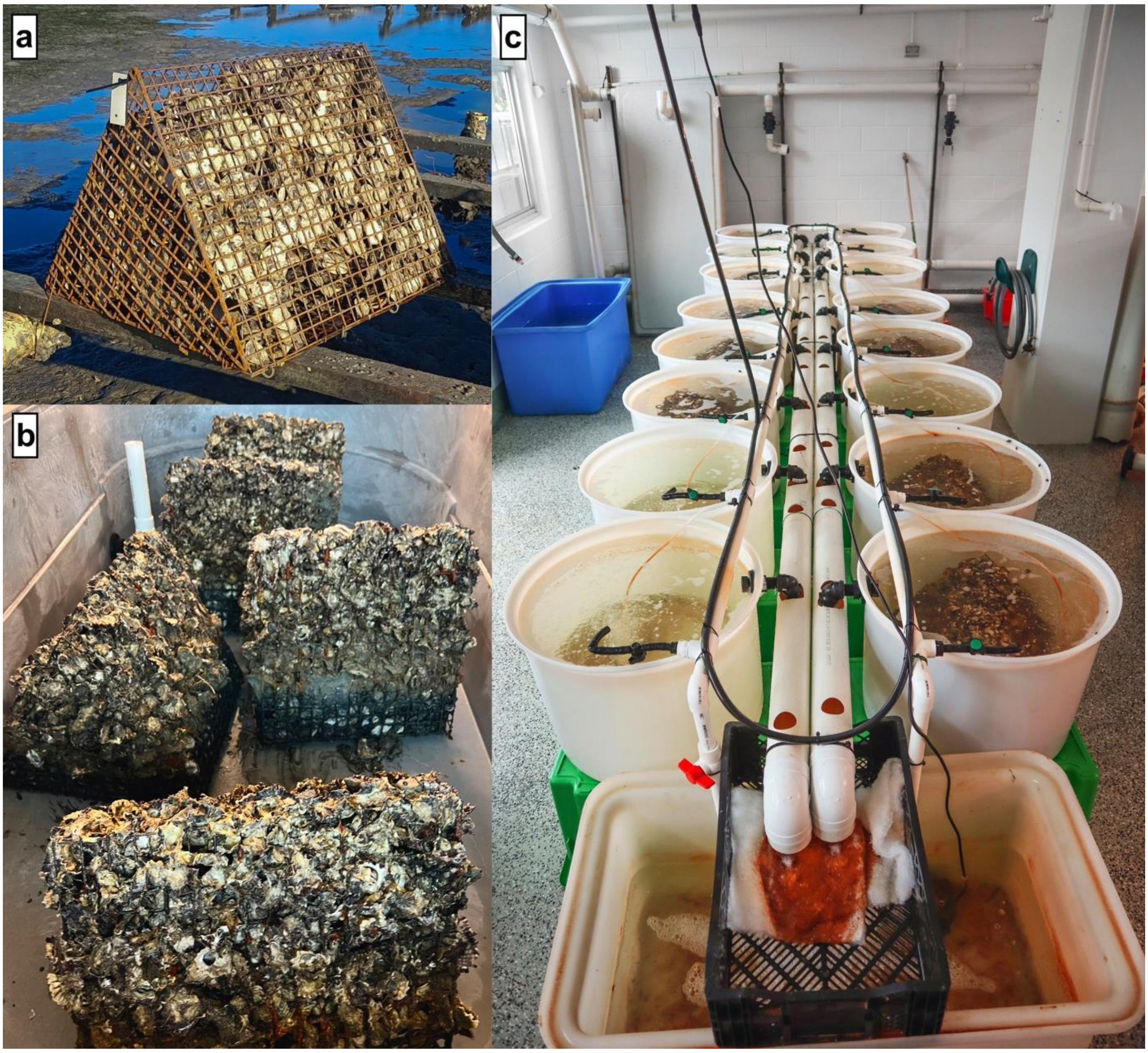

Fig. 1. (**a**) The ROB 400, before deployment in the intertidal oyster lease. Copyright: OzFish Unlimited (n.d.), (**b**) the ROB 400s after 19 months of deployment in an intertidal habitat. Copyright: Andersson (2023), and (**c**) the ex-situ experimental setup. Copyright: Andersson (2023).

Effect of light on clearances rates

To test whether clearance rates were different when exposed to light or dark, five ROB 400s tanks and three control tanks without ROB 400s were randomly assigned to a 'dark' treatment and covered with black polythene (GRUNT GRGB0042), while the remaining five ROB 400s tanks and three control tanks were assigned to a 'light' treatment and left uncovered exposed to constant light (LED 'cool white', mean 455.7 LUX ± 5.6 SD at the water surface, HOBO MX Temp/Light MX 2202). After 18 h, water flow was turned off and live *N. oceanica* was added to each tank at an initial mean density of 1,068,75 cells mL^{-1} ± 54,796 SD. Absorbance was measured as previously described and used to generate data on clearance rates for all replicates in the light and dark treatments as previously described.

Effect of temperature on clearance rates

We simulated a marine heatwave lasting a period of 5 days where mean temperatures were ~4 °C above ambient for that time of year (Hobday et al. 2016). The treatment was applied to five ROB 400s and three empty control tanks that were randomly allocated and heated by 3–4 °C using 300 W titanium aquarium heaters. Five ROB 400s and three empty control tanks were left at ambient temperatures of ~21 °C. Water flow was turned off after 24 h. Absorbance was measured after 24, 72, and 120 h as previously described, and used to generate data on clearance rates for the heated and ambient treatments as previously described.

Effect of salinity on clearance rates

To test the effects of a simulated flood, salinity was lowered with tap water (21.5 °C) to levels present during the 2010/2011 Queensland flood (Clementson et al. 2021; Oubelkheir et al. 2014); ambient salinity (36.2) in three ROB 400 tanks, ~15 salinity in three ROB 400 tanks and three control tanks, or ~25 salinity in four ROB 400 tanks and three control tanks. AquaOne Water Conditioner© ($Na_2S_2O_3$, H_2O) was added to all tanks (10 mL per tank). Water flow was turned off after 24 h. Absorbance was measured after 24, 72, and 120 h as previously described, and used to generate data on clearance rates for the reduced salinity and ambient treatments as previously described. After 120 h, salinity levels had increased slightly in all replicates due to evaporation (supplementary table S.2.B).

Statistical analysis

Data on clearance rates in the light/dark experiment were analysed by two-way ANOVA using ‘presence/absence of ROB 400’ and 'treatment’ as fixed factors, and tank as the level of replication. A type I sum of squares was used. Data on clearance rates in the simulated heatwave and flood experiments were analysed using repeated measures ANOVA design with ‘day’ as a random factor and ‘presence/absence of ROB 400’ and ‘treatment’ as fixed factors, respectively. Replicate (tank) was included in the model to account for non-independence of measurements taken from the same replicate over time. A type III sum of squares was used.

For all ANOVAs, assumptions of normality and heterogeneity of variance were examined using Q-Q residual plots, values for skewness and kurtosis, and Kolmogorov-Smirnov and Shapiro-Wilk tests in IBM SPSS v29.0 (Field 2018; Quinn and Keough 2002). Some data were not normally distributed, but as analyses done using transformed data gave the same outcomes as analyses of untransformed data, analyses done using untransformed data were presented. Significant outcomes ($p < .05$) with more than two levels were interrogated by splitting the interaction into multiple ANOVAs followed by Bonferroni post-hoc tests (Field 2018).

Results

Baseline clearance rates

After 90 min, the density of *N. oceanica* had decreased in tanks containing ROB 400s, but not in tanks without ROB 400s (Fig. 2a). The mean clearance rate of the tanks with ROB 400s present was 119.1 L h^{-1} ± 14.8 SE, though clearance rates were not consistent over time (Fig. 2b).

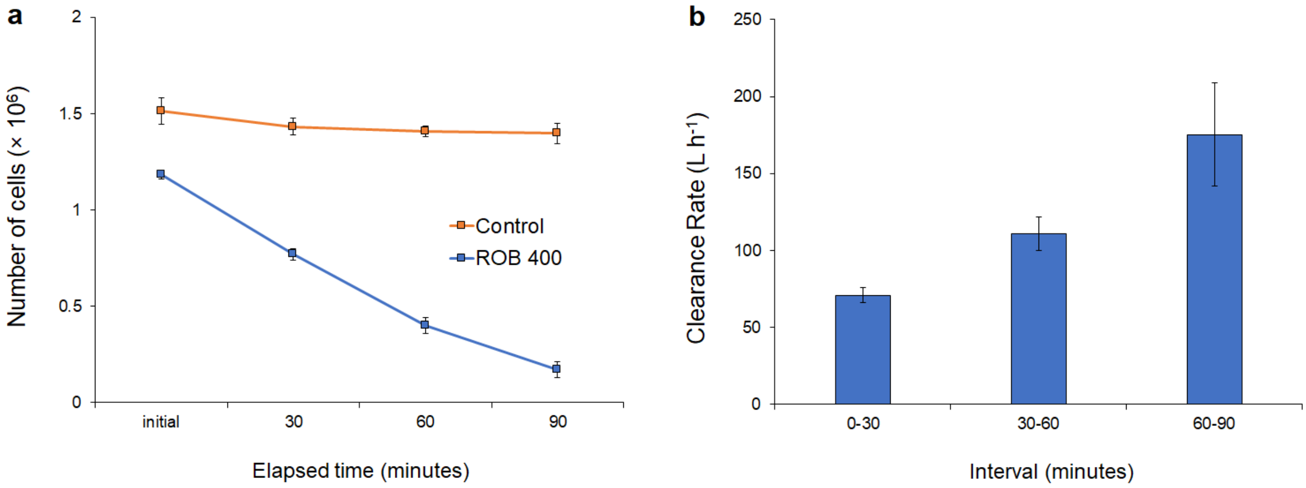

Fig. 2: Clearance of microalgae, *Nannochloropsis oceanica* (CS-246), by marine invertebrate communities living on modular oyster reef restoration baskets (ROB 400). **a.** Change in the density of *N. oceanica* in static, aerated tanks with (blue, ROB 400) and without (orange, Control) modular oyster reef restoration baskets over 90 minutes. Data are means ± SE, n = 9 for ROB 400, n = 5 for control. **b.** Clearance rates of invertebrate communities living on ROB 400 in each 30-minute interval over the 90-minute experiment. Data are means ± SE, n = 9.

Table 1: Outcomes of ANOVA analyses examining the effects of light, temperature, and salinity on the clearance rates of tanks with and without invertebrate communities living on modular oyster reef restoration baskets (ROB 400) in laboratory experiments. df, degrees of freedom; MS, mean square; p/a, presence/absence; temp, temperature. Significant factors are in bold ($p < .05$).

Parameters	Source	df	MS	F	*p*	Post hoc tests
Light	**presence/absence**	**1**	**1.130E5**	**325.38**	**<.0001**	present > absent
	treatment	1	3.70	0.01	.919	
	p/a × treatment	1	2.30E-2	<0.01	.994	
	error	12	347.29			
Temperature	**time**	**2**	**2.51E4**	**8.73**	**.002**	24 h = 120 h > 72 h
	presence/absence	**1**	**1.72E5**	**42.61**	**<.0004**	present > absent
	temperature	1	901.48	0.22	.644	
	p/a × temp	1	1.21E3	0.30	.594	
	p/a × time	2	533.35	0.19	.832	
	temp × time	2	1.34E3	0.47	.635	
	temp × time × p/a	2	2.99E3	1.04	.371	
	replicate (temp × p/a)	12	4.00E3	1.39	.248	
	error	20	2.87E3			
Salinity (Present)	**treatment**	**2**	**2.10E4**	**4.83**	**.029**	25 = 35 > 35 = 15
	time	2	203.94	0.47	.636	
	treatment × time	4	64.81	0.15	.960	
	replicate (treatment)	7	635.25	1.46	.268	
	error	12	433.98			
Salinity (Absent)	treatment	1	11.19	0.28	.613	
	time	**2**	**325.66**	**8.05**	**.012**	24 h = 120 h > 120 h = 72 h
	treatment × time	2	0.99	0.25	.976	
	replicate (treatment)	4	37.50	0.93	.494	
	error	8	40.44			

Effect of light on clearance rates

There was no significant effect of light on the clearance rates of tanks containing ROB 400s (Fig. 3, Table 1). Tanks with ROB 400s present had significantly higher clearance rates than control tanks without ROB 400s (Table 1; present > absent).

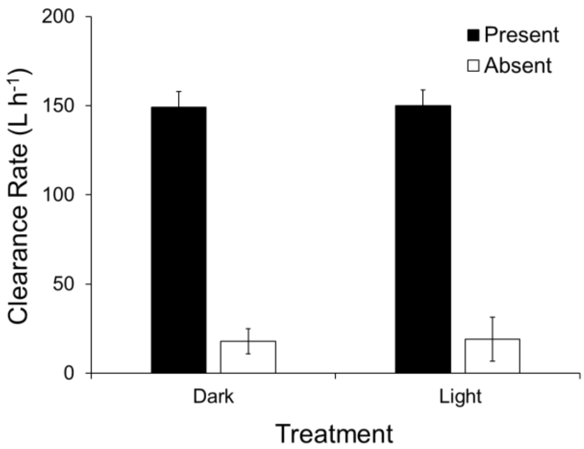

Fig. 3: Effects of presence/absence of modular robust oyster baskets (ROB 400) and light on clearance rates measured in static aerated tanks. Tanks were held in darkness or light (mean 455.70 LUX ± 5.60 SD) for 18 h before being fed *Nannochloropsis oceanica* CS-246 at a density of ~1 × 10^6 cells mL^{-1}. Clearance rates were affected by the presence/absence of ROBs, but were not influenced by treatment nor the interaction between these factors (Table 1). Data are means ± SE; n = 5 for present, n = 3 for absent.

Effect of temperature on clearance rates

An increase in temperature (~4 °C) had little effect on clearance rates of ROB 400s over five days (Fig. 4, Table 1). Tanks with ROB 400s present had significantly higher clearance rates than control tanks without ROB 400s (Table 1; present > absent). Clearance rates fluctuated over time, with clearances rates measured at 72 h lower than at other times (24 = 120 > 72 h) (Fig. 4, Table 1). There were no significant interactions among factors (Table 1).

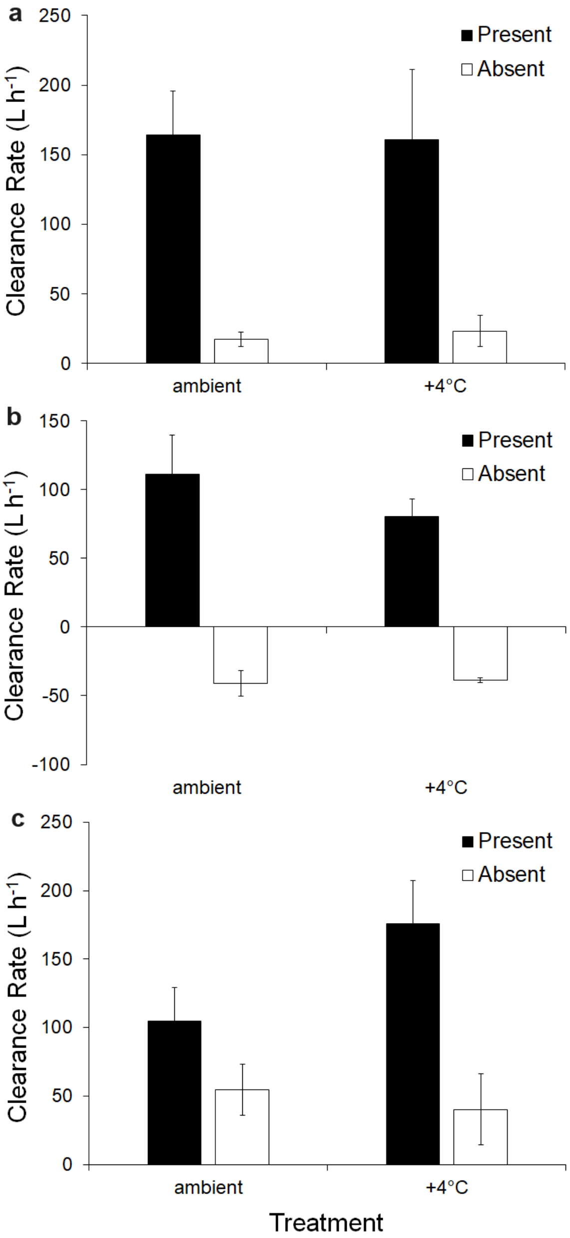

Fig. 4: Effects of presence/absence of modular robust oyster baskets (ROB 400) and temperature on clearance rates measured in static aerated tanks. Tanks were held at ambient (~21 °C) and warmed (+4 °C, ~25 °C) temperatures for **a**. 24h, **b**. 72 h, and **c**. 120 h before being fed *Nannochloropsis oceanica* CS-246 at a density of ~1–1.5 × 10^6 cells mL^{-1}. Clearance rates were affected by the presence/absence of ROBS (present > absent) and varied across time (24 h = 120 h > 72 h), but were not influenced by temperature nor any interaction between these factors (Table 1). Data are means ± SE; *n* = 3–5 for present, *n* = 3 for absent.

Effect of salinity on clearance rates

In tanks containing ROB 400s, decreases in salinity had a significant effect on clearance rates (Fig. 4, Table 1). Outcomes showed an overlapping hierarchy of significance with clearance rates higher in 25 salinity treatment than in the 15 salinity treatment, but the ambient treatment had the same clearance rates as both the 25 and 15 treatments (Table 1; 25 = 36 > 36 = 15 salinity). In tanks without ROB 400s, there was no effect of salinity, but clearance rates fluctuated over time (24 = 120 > 120 h = 72 h) (Fig. 4, Table 1).

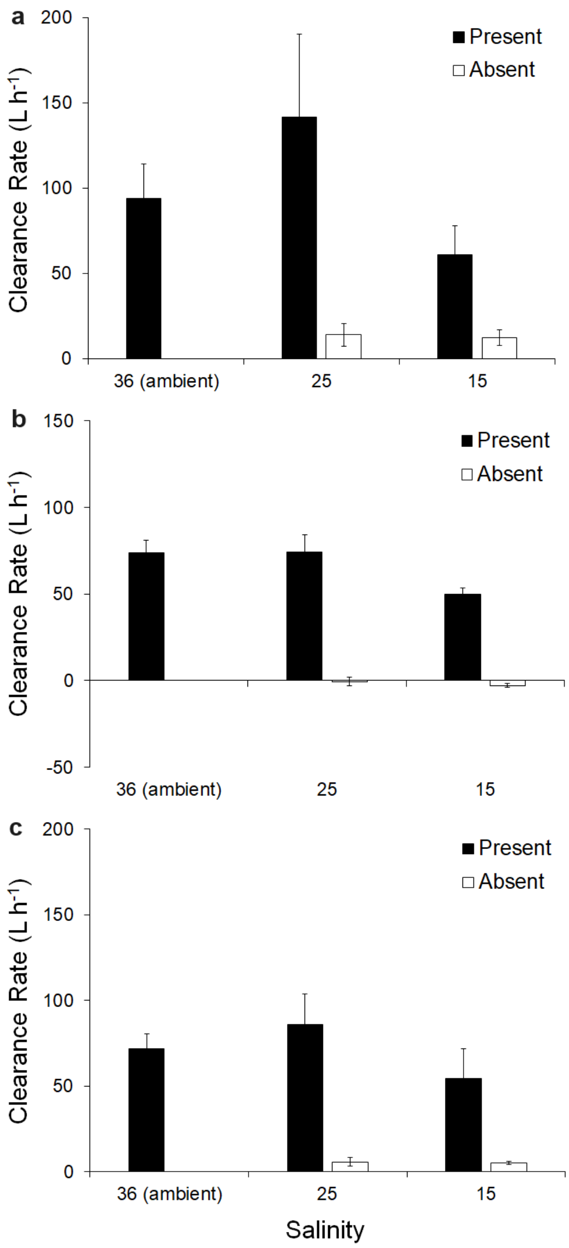

Fig.5: Effects of presence/absence of modular robust oyster baskets (ROB 400) and salinity on clearance rates measured in static, aerated tanks. Tanks were held at ambient salinity (~36) or reduced salinity (~25 or ~15) for **a**. 24h, **b**. 72 h, and **c**. 120 h before being fed *Nannochloropsis oceanica* CS-246 at a density of ~1.5 × 10^6 cells mL^{-1}. Data unavailable for the ambient-absent treatment at all times. Clearance rates for tanks with (present) and without (absent) ROB 400s were analysed separately (Table 1). Clearance rates when ROB 400s were present were affected by salinity (25 = 35 > 35 = 15) but were not influenced by time nor any interaction between these factors (Table

1). Clearance rates when ROB 400s were absent varied across time (24 h =120 h > 120 h = 72 h) but were not influenced by salinity nor any interaction between these factors (Table 1). Data are means ± SE; n = 3 for present except for present-25 where n = 4; n = 3 for absent.

Discussion

Baseline clearance rates of invertebrate communities on ROB 400s were 119.06 L h^{-1}. It is difficult to directly compare our results with the results of previous studies due to differences in methods used, but our values are generally similar to values for communities dominated by oysters and mussels. For instance, bivalve beds dominated by *Crassostrea gigas* and *Mytilus edulis* had clearance of 138.6 ± 32.7 l h^{-1} m^{-2} (n = 18) and 447.2 ± 97.8 l h^{-1} m^{-2} (n = 16), respectively (Vismann et al. 2016). In a mixed bivalve bed, clearance rates increased from 193.5 to 806.1 L $h^{-1}m^{-2}$ after algae enrichment (Hansen et al. 2011). Invertebrate communities living on ROB 400s increased their clearance rates through time, a trend also observed for bivalves (e.g., Gatenby et al. 2013). One explanation for this could be compensatory food intake where filter feeders increase the amount of water they pass though their bodies as algae concentrations decrease (Bayne et al. 1987; Bayne et al. 1993; Barillé et al. 1993).

We found no effect of light on clearance rates of invertebrate communities occupying ROB 400s. We are not aware of any other study that has tested the effects of light on a community of marine filter feeders, but studies done on individual species indicate that the effects of light on clearance rates is species specific (Pouil et al. 2021). For instance, light limitation led to increases in clearance rates of *Corbicula fluminea* (110 ± 15 mL g^{-1} h^{-1}), but not *Utterbackia imbecillis* (24 ± 6 mL g^{-1} h^{-1})(Hills et al. 2020). The negligible impact of light in this study might be because communities were dominated by intertidal oysters. Intertidal species are generally less affected by light because they must feed while they are submerged to gain adequate nutrition, regardless of the time of day (Loosanoff and Nomejko 1946).

We found no effect of an increase in temperature on clearance rates of invertebrate communities. This contrasts with studies performed on single bivalve species which found that clearance rates generally increase with temperature until they reach their thermal limit (e.g., Ren et al. 2000; Yukihira et al. 2000; Carneiro et al. 2020). One explanation for why our results differ could be that the bivalve communities we tested were adapted to an intertidal environment where they are regularly exposed to a broad range of temperatures. We also mimicked a heatwave occurring during winter/early spring. An increase in temperature of 4 °C would likely have more of an impact during summer months when the invertebrates would be closer to their upper temperature threshold.

Clearance rates were generally reduced when salinity was lowered from ~25 to ~15, perhaps because the invertebrates living in the ROB 400s approached their tolerance limit (McFarland et al. 2013) at the lower extremes of salinity these invertebrate communities experience in nature (Clementson et al. 2021, Oubelkheir et al. 2014). Our results are similar to previous studies that measured clearance rates of marine filter feeders using similar exposure levels (e.g., Navarro and Gonzales 1998; Casas et al. 2018). Our results indicate invertebrate communities living on restoration structures show signs of stress when exposed to very low salinity but may continue to provide substantial filtration services regardless. As clearance rates were highest at a salinity of ~25, oyster reef restoration may be as effective in inland coastal waterways, where the salinity is usually lower, than in the ocean.

Across all experiments, we observed density of microalgae varied in the control tanks. This highlights the importance of using controls for accurate evaluation of filtration rates. To date, most studies investigating bivalve clearance include controls only when performed in a laboratory setting. Filtration studies on community bivalve functions performed *in situ* generally lack controls (e.g., Hansen et al. 2011). We suggest future studies should include controls to avoid over-estimating the clearance rates of filter-feeding invertebrate communities.

This study demonstrates modular oyster baskets can be used to test the effects of light, temperature, and salinity on clearance rates of invertebrate communities that colonise reef restoration structures. Modular baskets bypass common challenges presented during filtration studies on bivalves, both *ex situ* (e.g., high level of disturbance, small volumes, restriction to study few animals and often single species) and *in situ* (e.g., dependence on weather and tide, inability to manipulate physiochemical parameters, lack of controls, poor replication). Future studies are recommended to expand our baseline study by, for instance, evaluating the clearance rates of the invertebrate communities in a wider range of experimental conditions predicted for Moreton Bay over the next century (e.g., lower pH, reduced oxygen levels, increased turbidity), and to test identical and more extreme conditions over extended periods as extreme events often last beyond the 5 days tested here (e.g., summer heatwave or reduced salinity over weeks).

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Supplementary information A

Relationship between absorbance and cell density for N. oceanica

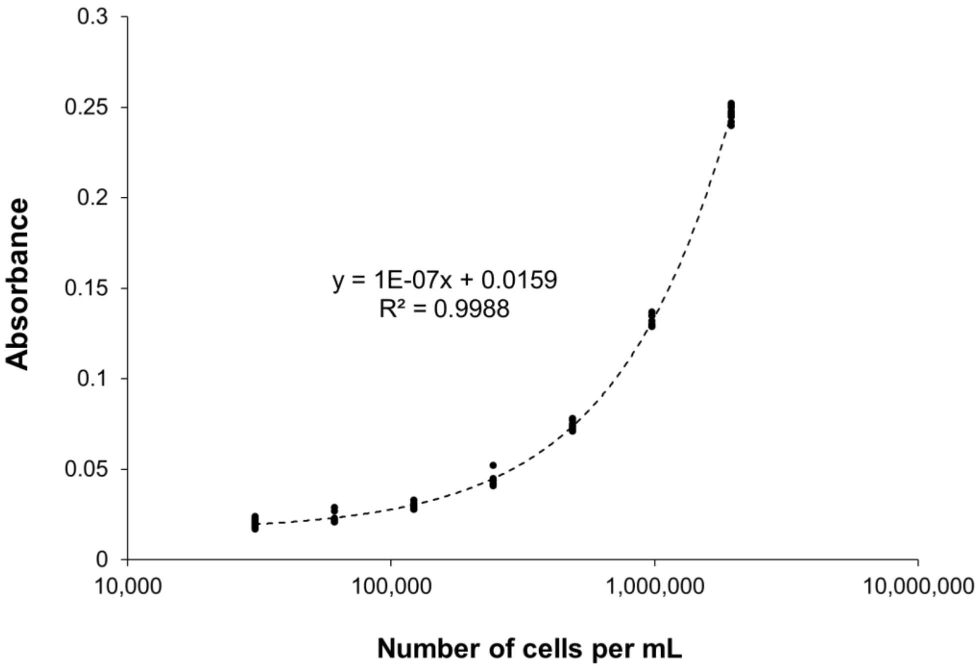

Fig. S.A.1: Calibration curve of cell density for *Nannochloropsis* (log) at different concentrations (1.5215×10^1–1.9475×10^6 cells/mL) against absorbance.

Supplementary information B

Experimental setup for each replicate

Table S.B.1: The randomly allocated setup for each replicate (ROB 400/Control, volume in each tank (L), weight of the ROB 400 if present (kg), and treatment type (light/dark, heated/ambient and salinity).

Replicate nr. #	ROB/Control	Volume of seawater in each tank (L)	Weight of ROB 400 (kg)	Light/dark	Heated/ Ambient	Salinity
1	Control	107	–	Light	Ambient	15
2	Control	107	–	Dark	Ambient	25
3	ROB 400	81.5	43.7	Dark	Ambient	15
4	ROB 400	80.0	42.7	Light	Heated	36
5	ROB 400	80.5	43.4	Dark	Heated	36
6	ROB 400	80.0	42.2	Dark	Ambient	25
7	Control	107	–	Light	Heated	25
8	ROB 400	80.0	40.7	Light	Heated	25
9	ROB 400	82.0	42.1	Dark	Ambient	25
10	Control	107	–	Dark	Heated	15
11	Control	107	–	Light	Ambient	15
12	ROB 400	83.0	38.5	Light	Heated	35
13	ROB 400	85.5	39.9	Light	Ambient	25
14	Control	107	–	Dark	Heated	25
15	ROB 400	81.0	41.5	Dark	Heated	15
16	ROB 400	81.0	39.1	Light	Ambient	15

418 *Table of water quality parameters for each treatment*

419 **Table S.B.2:** Average water parameters (temperature (°C), salinity and dissolved oxygen (mg/L)) for
420 the temperature and salinity treatments before performing the experiments with the standard deviation
421 in parenthesis.

ROB 400/Control	Treatment	Elapsed time (h)	Average temperature (°C)	Average salinity	Average dissolved oxygen (mg/L)
ROB 400	+4°C	24	24.072 (0.173)	–	–
Control	+4°C	24	24.697 (0.738)	–	–
Rob 400	Ambient temp.	24	21.906 (0.116)	–	–
Control	Ambient temp.	24	21.713 (0.164)	–	–
ROB 400	+4°C	72	24.092 (0.184)	–	–
Control	+4°C	72	24.13 (0.765)	–	–
ROB 400	Ambient temp.	72	19.542 (0.164)	–	–
Control	Ambient temp.	72	19.413 (0.135)	–	–
ROB 400	+4°C	120	23.976 (0.22)	39.46 (0.108)	4.752 (0.307)
Control	+4°C	120	24.287 (0.769)	38.933 (0.176)	4.707 (0.21)
ROB 400	Ambient temp.	120	19.3 (0.14)	38.34 (0.204)	6.924 (0.46)
Control	Ambient temp.	120	19.067 (0.278)	37.833 (0.318)	6.927 (0.799)
ROB 400	15 salinity	24	17.51 (1.812)	15.50 (0.50)	6.907 (0.155)
Control	15 salinity	24	21.04 (0.046)	15.10 (0.058)	7.17 (0.349)
ROB 400	25 salinity	24	20.938 (0.038)	25.55 (0.185)	6.043 (0.201)
Control	25 salinity	24	20.983 (0.06)	24.733 (0.186)	7.117 (0.069)
ROB 400	35 salinity	24	20.977 (0.028)	35.70 (0.321)	6.017 (0.113)
ROB 400	15 salinity	72	21.117 (0.02)	15.80 (0.115)	7.05 (0.387)
Control	15 salinity	72	20.843 (0.103)	15.667 (0.088)	7.963 (0.718)
ROB 400	25 salinity	72	20.985 (0.043)	26.025 (0.202)	6.243 (0.32)
Control	25 salinity	72	20.977 (0.075)	24.833 (0.133)	6.557 (0.378)
ROB 400	35 salinity	72	20.96 (0.047)	36.366 (0.088)	6.33 (0.307)
ROB 400	15 salinity	120	21.53 (0.01)	16.767 (0.088)	7.297 (0.206)
Control	15 salinity	120	21.243 (0.10)	16.067 (0.145)	7.753 (0.15)
ROB 400	25 salinity	120	21.418 (0.062)	26.05 (0.26)	7.100 (0.303)
Control	25 salinity	120	21.353 (0.058)	25.31 (0.19)	6.537 (1.118)
ROB 400	35 salinity	120	21.347 (0.032)	36.833 (0.033)	5.937 (0.143)

422