Quantifying clearance rates of restored shellfish reefs using modular baskets

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Abstract

Shellfish reefs are among the most degraded ecosystems globally, prompting substantial efforts to restore them. While biodiversity gains of restored reefs are well documented, other ecosystem services such as water filtration remain poorly quantified. We present a novel way of measuring water filtration by restored reefs using modular restoration structures called Robust Oyster Baskets (ROB 400). Ten ROB 400s, colonised by mixed invertebrate communities over 19 months in Moreton Bay, Australia, were retrieved and placed individually in tanks within a recirculating seawater system. We first obtained baseline clearance rates by measuring changes in the density of microalgae *Nannochloropsis oceanica* (CS-246) over 1.5 h in tanks with and without ROB 400s. Mean clearance rates per ROB 400 were 119.1 L h^{-1} ± 14.8 SE. Second, we tested how clearance rates in tanks with and without ROB 400s were affected by (1) light/dark by covering tanks with black polyethylene, (2) temperature by heating half of the total number of tanks ~4 °C above ambient for five days, and (3) reduced salinity by addition of freshwater from ambient (~36) to ~25 or ~15 respectively. Clearance rates were reduced by ~45% when the salinity was ~15 compared to ~25 but were not affected by light *vs* dark, or temperature (ambient *vs* +4 °C). Our results demonstrate modular restoration structures can be used to quantify ecosystem services provided by restored reefs and to assess the vulnerability of natural and restored shellfish communities to current and future threats such as light pollution, heatwaves, and floods.

Introduction

Shellfish reefs form when living bivalves aggregate on hard 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 shellfish reefs are gone, fallen to overharvesting, habitat loss, pollution, and disease (reviewed by Beck et al. 2011; Gilby et al. 2018; Gillies et al. 2018). In North America, Europe, and Australia, several oyster reef habitats are functionally extinct (Beck et al. 2011; Gillies et al. 2020). Numerous efforts are underway globally to restore shellfish reefs using artificial constructions of recycled shell, crushed concrete, or natural rock (Coen and Luckenbach 2000; Hernández et al. 2018; McAfee et al., 2022).

Most studies that have investigated the efficacy of artificial structures in restoring shellfish reefs measured changes in biodiversity (e.g., Gilby et al. 2019; Xu et al. 2023). The capacity of artificial reefs to restore other ecosystem services, such as water filtration, remains less clear. One reason for this is the difficulty of measuring ecosystem services *in situ*. Current methods to measure the amount of water filtered by organisms (hereafter clearance rates) in the field can be large and heavy, are not easily replicated, are impractical for manipulative experiments, vulnerable to tide and weather, confined to shallow water, expensive, and/or limited to short deployment periods (Riisgård 2001; Grizzle et al. 2008; Hansen et al. 2011). Some studies have attempted to overcome these limitations by measuring clearance rates by bivalves in the laboratory (e.g., Castle and Waltham 2022; Cottingham et al. 2023) or in the field using devices tailored to specific situations and species (Riisgård 2001; Galimany et al. 2011). However, extrapolations from studies on single species and individuals generally overestimate the clearance rates of invertebrate communities by not accounting for inactive individuals (i.e., non-feeding) and interspecific or intraspecific interactions (Hansen et al. 2011). Additional approaches that overcome shortcomings in methods used to measure clearance rates by restored reefs are needed.

Some shellfish reef restoration projects are deploying modular restoration structures to provide new habitat for wild animals or protection from predators for seeded spat, juveniles, or adult shellfish (e.g., Walters et al. 2022; Grizzle et al. 2024). In Australia, the not-for-profit organisation OzFish Unlimited designed a modular system called Robust Oyster Baskets (hereafter ROB 400) and deployed ~5000 units in Moreton Bay/Quandamooka, near Brisbane on Australia’s east coast from 2018 (Fig. 1). Compared to restoration methods that pile rock or shell to create shellfish ‘beds’, modular restoration structures are easy to deploy and retrieve, can be deployed in complex configurations including hanging designs, provide protection against predation, more easily facilitate public participation, and can be tailored to suit the needs of each restoration project (pers. obs.; also see Walters et al. 2022; Grizzle et al. 2024). In this study, through real-world application we demonstrate that modular shellfish reef restoration structures can also be used to provide baseline data on clearance rates of invertebrate communities colonising shellfish reef restoration structures and for manipulative experiments measuring community responses to stressors.

Shellfish reef restoration projects occur amid ongoing human impacts (Halpern et al. 2008; Babcock et al. 2019). To ensure effective and enduring restoration, understanding of the ways in which restored shellfish reefs respond to stressors is crucial. While single species studies show bivalve molluscs feed less when heat stressed, predicting the effects of temperature changes on whole shellfish communities remains challenging due to interacting direct and indirect effects (Turner et al. 2016; Cole et al. 2021). Freshwater influxes negatively affect bivalve growth, survival, and recruitment (Pourmozaffar et al. 2020) and reduce biodiversity on temperate restored shellfish reefs (Marshall et al. 2019). Floods are expected to become more intense and frequent (Allen and Ingram 2002), and the consequences of shifting freshet regimes for restored shellfish reefs is uncertain (Marshall et al. 2019). The influence of recently recognised stressors on restored shellfish reefs, such as light pollution (Davies et al. 2014), is poorly understood. Many marine shellfish can detect and respond to light (Audino et al. 2020) and may depend on natural light cycles for successful functioning (García-March et al. 2008; Gnyubkin 2010). Studies examining the impacts of light pollution on shellfish reefs are lacking (but see Christoforou et al. 2023).

Current approaches to measuring clearance rates by shellfish reefs are not readily adapted to testing the impacts of stressors. *In situ* methods may not be practical for manipulative experiments because of short deployment periods, the inherent difficulties of working in the field, and high costs (Riisgård 2001; Grizzle et al. 2008; Hansen et al. 2011). While small-scale laboratory approaches are useful for quantifying the impacts of stressors (Benton et al. 2007), outcomes of manipulative experiments on single species may not accurately represent the responses of assemblages to the same stressor/s (Wernberg et al. 2012). We propose modular shellfish structures can be used to reveal the vulnerability of shellfish reefs to stressors. To demonstrate this, we examined how clearance rates of shellfish-dominated invertebrate communities living on ROB 400s were affected by different levels of light, temperature, and salinity in single-factor experiments.

Materials and Procedures

Robust Oyster Basket 400

Robust Oyster Baskets (ROB 400s) have a triangular prism-shaped design (400 × 400 × 300 mm) made of mild steel welded wire mesh (2.5 mm wire, 25 × 25 mm aperture) encasing recycled oyster shells (Fig. 1a) which, after deployment, are colonised naturally by mixed invertebrate communities dominated by filter-feeding bivalves (particularly rock oysters) (Fig. 1b). Ten ROB 400s that had been positioned intertidally were collected (July 2023) after 19 months of deployment in Moreton Bay/Quandamooka, Queensland, Australia (-27.45684, 153.39528), transported out of water, and then housed individually in aerated seawater in 113-L polyethylene tanks (white colour, 455 mm H, 637 mm diameter, Nally Plastics IPO25) at The University of Queensland's Moreton Bay Research Station, Minjerribah (North Stradbroke Island), Queensland, Australia from July to December 2023 (Fig. 1c). Seawater at ambient temperature (35–37 salinity, ~21 °C) was recirculated among ten tanks containing ROB 400s, six tanks without ROB 400s, 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 [Cell-hi F2P, Varicon aqua], 25 °C, ~35 salinity, 22:2 h light/dark photoperiod) and Shellfish Diet 1800 (Reed Mariculture) three times per week. Seawater (50–66%) was exchanged monthly. When ROBs were added to the system, ammonia levels spiked to 0.5–1.0 ppm (API Ammonia NH_3/NH_4^+ test kit) but fell below 0.5 ppm within 3 days. Thereafter ammonia was undetectable (<0.25 ppm). Temperature, salinity, and dissolved oxygen (DO) were monitored with a Horiba U-52 Series MultiParameter Water Quality Meter. Values recorded during experiments are reported in Supporting Information Table S1. ROB 400s were left undisturbed for at least 14 days between experiments. The surface area colonised by invertebrates, the number of living and dead filter feeding invertebrates (>10 mm), and the height and length (*sensu* Galtsoff 1964) of 20 haphazardly selected rock oysters (the dominant invertebrates on ROB 400s) were measured using a tape measure, by visual count, and vernier callipers (± 1.0 mm), respectively, 14 days after the final (salinity) experiment (Supporting Information Table S2).

Baseline clearance rates

To quantify baseline clearance rates, we measured changes in the density of live 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 ten independent tanks housing ROB 400s and six independent tanks without ROB 400s. Each tank was continuously aerated to maintain DO levels and keep microalgae in suspension. Live *N. oceanica* were added to each tank at an initial mean density of 1.3×10^6 cells $mL^{-1} \pm 5.2 \times 10^4$ SD. Total absorbance (sum of $\lambda = 750, 664, 647, 630$ nm) was measured from one sample taken from each replicate tank 40 min after microalgae were added and every 30 min thereafter with a spectrophotometer (Hach DR 5000™ UV-Vis, Starn Pty Ltd glass cuvette, Type 1, match code 7, path length 10 mm) until 2 h had elapsed or the density of the microalgae fell below $\sim 9 \times 10^4$ cells mL^{-1}. Cuvettes were triple rinsed between samples, and samples were directly pipetted from the respective tank (~100–150 mm from the edge of the tank and ~30–50 mm depth). Data on the density of *N. oceanica* in each tank at each time point were derived from total absorbance data (Supporting Information Fig. S1). Clearance rates were then calculated for each replicate per Eq. 1 modified from Riisgård (2001):

$$\boldsymbol{Cl} = (\boldsymbol{V}/\boldsymbol{t})\ \boldsymbol{ln}(\boldsymbol{C_0}/\boldsymbol{C_t}) \qquad (1)$$

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 (Supporting Information Table S2).

Effect of light on clearances rates

Moreton Bay/Quandamooka is one of the most light polluted coastal regions in Oceania, likely exposing restored shellfish communities to artificial light at night directly and via ‘skyglow’ (Kamrowski et al. 2012; Davies et al. 2014). To test whether clearance rates by invertebrate communities were different when exposed to extended periods of light or dark, five ROB 400s tanks and three control tanks without ROB 400s were randomly assigned to a ‘dark’ treatment and covered (top and sides) with black polythene (GRUNT GRGB0042, mean 5.1 lux ± 2.6 SD at the water surface, HOBO MX Temp/Light MX 2202 set to record every 10 min), 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 set to record every 10 min). After 18 h, water flow was turned off and live *N. oceanica* were added to each tank at an initial mean density of 1.09×10^6 cells $mL^{-1} \pm 5.5 \times 10^4$ SD.

Absorbance was measured at the beginning of the experiment and every 30 min thereafter as previously described and used to derive clearance rates for all replicates in the light and dark treatments for the period 0–60 min after microalgae was added.

Effect of temperature on clearance rates

Moreton Bay/Quandamooka is on Australia's east coast in a global climate change hotspot, and forms part of the 45% of Australia's coastline which is experiencing unprecedented numbers of extreme climate events (heatwaves, cyclones, floods, drought, low sea level) which are expected to further intensify and become more frequent in future (Hobday et al. 2016; Babcock et al. 2019). We simulated a marine heatwave lasting a period of 5 days where mean temperatures were ~4 °C above ambient for that time of year (*sensu* Hobday et al. 2016). The heatwave treatment was applied to five randomly allocated tanks containing ROB 400s and three randomly allocated control tanks without ROB 400s that were heated by 3–5 °C using 300 W titanium aquarium heaters (Aqua One TH300 or Aqualogic). Five tanks with ROB 400s and three control tanks without ROB 400s were left at ambient temperatures of ~20 °C. Water flow was turned off when heaters were added to the tanks (time 0) and remained off for the duration of the experiment. After 24 and 120 h, absorbance was measured when *N. oceanica* was added and every 30 min thereafter as previously described and used to derive clearance rates for all replicates in the heatwave and ambient treatments for the period 0–60 min after microalgae was added. Temperature, salinity, and DO were monitored with a Horiba U-52 Series MultiParameter Water Quality Meter (Supporting Information Table S1).

Effect of salinity on clearance rates

To test the effects of a simulated low salinity event, salinity was lowered with tap water (21.5 °C, <0.01 salinity) in randomly allocated tanks to levels recorded in Moreton Bay during the most recent major low salinity event, the 2010/2011 Brisbane River flood (Oubelkheir et al. 2014; Clementson et al. 2021); ~15 salinity in three tanks containing ROB 400s and three control tanks without ROB 400s, or ~25 salinity in four tanks containing ROB 400s and three control tanks without ROB 400s. Salinity was not altered in three tanks containing ROB 400s (salinity 36.2). AquaOne Water Conditioner© ($Na_2S_2O_3$, H_2O) was added to all tanks (10 mL per tank). Water flow to all tanks was turned off before the freshwater was added (time 0) and remained off for the duration of the experiment. After 24, 72, and 120 h, absorbance was measured when *N. oceanica* was added and every 30 min thereafter as previously described and used to derive clearance rates for the reduced salinity and ambient treatments for the period 0–120 min after microalgae was added. Temperature, salinity, and DO were monitored with a Horiba U-52 Series MultiParameter Water Quality Meter (Supporting Information Table S1). After 120 h, salinity levels had increased by ~1 in all tanks due to evaporation (Supporting Information Table S1).

Statistical analysis

For the baseline study, data on algal density in tanks with and without ROB 400s were analysed separately using repeated measures ANOVA design with ‘time/interval’ as a fixed factor. Replicate (tank) was included in the model to account for non-independence of measurements taken from the same replicate over time. A Type I sum of squares was used. Data on clearance rates in the light 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 III sum of squares was used. Data on clearance rates in the temperature experiment were analysed using repeated measures ANOVA design with ‘day’, ‘presence/absence of ROB 400’, and ‘temperature’ as fixed factors. Replicate (tank) was included in the model to account for non-independence of measurements taken from the same replicate over time. A Type I sum of squares was used. For the salinity experiment, replicates with ROB 400s and replicates without ROB 400s were analysed separately. Data on clearance rates were analysed using repeated measures ANOVA design with ‘day’ and ‘treatment’ as fixed factors. Replicate (tank) was included in the model to account for non-independence of measurements taken from the same replicate over time. A Type I sum of squares was used.

All ANOVAs were done using the permutational analysis of variance (PERMANOVA) routine in Primer v7.0.23 software package. Pair-wise comparisons of untransformed data were generated using Euclidean distance and 9999 permutations of the raw data. Significant outcomes ($p < .05$) with more than two levels were interrogated by pair-wise tests. Monte-Carlo values were used when there were fewer than 100 permutations. 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 (Quinn and Keough 2002; Field 2018). Data for the salinity experiment with ROBS present were not normally distributed, but as normality is not an assumption for PERMANOVA (Anderson 2017), untransformed data were analysed. All other data met the assumptions of traditional ANOVA. Any outliers were included in analyses as exclusion had no effect on the outcomes.

Assessment

Baseline clearance rates

Density of *N. oceanica* fell in tanks containing ROB 400s (Fig. 2a), decreasing by almost 85% after 90 min (Fig. 2b). In contrast, the density of *N. oceanica* in control tanks without ROB 400s remained stable (Fig. 2a) and was not significantly different at the final measurement (130 min) compared to the initial measurement (repeated measures ANOVA, $F_{1,9} = 0.03$, $p = .204$, Fig. 2b). The mean clearance rate of tanks with ROB 400s present was 119.1 L h^{-1} ± 14.8 SE, though clearance rates were not consistent over time (Fig. 2c). For instance, mean clearance rates were initially 70.8 L h^{-1} ± 4.8 SE but more than doubled to 175.4 L h^{-1} ± 33.4 SE in the period from 100–130 min after *N. oceanica* were added (repeated measures ANOVA, $F_{2,24} = 6.63$, $p = .004$, followed by pairwise test: *40-70 < 70-100 < 100-130*, Fig. 2c).

Effect of light on clearance rates

There was no 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).

Effect of temperature on clearance rates

An increase in temperature of ~4 °C had no effect on clearance rates of the invertebrate communities living on 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 did not significantly vary between day 1 and day 5 (Fig. 4, Table 1), and there were no significant interactions among any factors (Table 1).

Effect of salinity on clearance rates

In tanks containing ROB 400s, salinity had a significant effect on clearance rates (Fig. 5, Table 1). Post hoc pair-wise tests (Table 1) indicated there was an overlapping hierarchy of significance, with clearance rates higher in the 25 salinity treatment than in the 15 salinity treatment, but clearance rates in the ambient treatment were not significantly different than either the 25 or 15 salinity treatments (Table 1; 25 = ambient > ambient = 15). In tanks without ROB 400s, salinity had no effect, but the density of the algae behaved in different ways depending on the day, with no significant interactions among factors (Fig. 5, Table 1, 1 = 5 > 5 = 3 days). On day 1, mean clearance rates in tanks without ROB 400s were positive (i.e. >0, Fig. 5a) as the density of *N. oceania* decreased, perhaps due to cells clumping together or falling out of suspension. However, on day 3, mean clearance rates in tanks without ROB 400s were negative (i.e., <0, Fig. 5b) as the density of the *N. oceania* increased, perhaps due to reproduction or disaggregation of cells. On day 5, mean clearance rates in tanks without ROB 400s were also positive (i.e. >0, Fig. 5c), but were not significantly different than clearance rates at either day 1 or day 3.

Discussion

In ambient conditions, mean clearance rates by invertebrate communities on ROB 400s varied between 251.0 and 522.6 L h^{-1} m^{-2} among experiments. These values are similar to clearance rates reported for invertebrate communities dominated by oysters and mussels in field studies (e.g., Hansen et al. 2011; Vismann et al. 2016; Rullens et al. 2023). For instance, bivalve beds dominated by *Crassostrea gigas* and *Mytilus edulis* had clearance rates 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 mussel-dominated shellfish bed, clearance rates increased from 193.5 to 806.1 L h^{-1} m^{-2} after microalgae were added (Hansen et al. 2011). We also found rock oyster-dominated invertebrate communities living on ROB 400s increased their clearance rates through time, a trend also observed for freshwater rainbow mussels, *Villosa iris*, fed a high food ration (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; Barillé et al. 1993; Bayne et al. 1993). Our results demonstrate modular restoration structures can be used to obtain data on clearance rates of whole communities *ex situ* without the inherent difficulties of sampling in the field and over-estimation of clearance rates extrapolated from measurements on individuals (Hansen et al. 2011). With further refinement and validation of our approach, modular restoration structures could enable restoration programs to monitor clearance rates easily and cheaply, facilitating access to national and international programs that fund verifiable ecosystem services.

We found no effect of light on clearance rates of invertebrate communities occupying ROB 400s. Our results are similar to those of Christoforou et al. (2023) who reported no effect of ALAN (artificial light at night) on the total proportion of phytoplankton consumed by the mussel *Mytilus edulis*. We are not aware of any other study that has tested the effects of light on clearance rates by a community of marine filter feeders, but studies done on freshwater mussels indicate that the effects of light on clearance rates is likely species specific (Hills et al. 2020; Pouil et al. 2021). For instance, exposure to darkness led to increased clearance rates by the Asian clam, *Corbicula fluminea*, but not by the paper pondshell, *Utterbackia imbecillis* (Hills et al. 2020). The negligible impact of light in this study might be because communities were dominated by intertidal rock 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 living on ROB 400s. This contrasts with studies performed on single bivalve species which found that clearance rates generally increase with temperature until a thermal limit is reached beyond which feeding is depressed and clearance rates rapidly decline (e.g., Ren et al. 2000; Yukihira et al. 2000; Parker et al. 2024). One explanation for why our results differ from those of previous studies could be that the invertebrate communities we tested were adapted to an intertidal environment where they are regularly exposed to a broad range of temperatures (Potter and Hill 1982; Helmuth et al. 2006). We also simulated a marine heatwave occurring during winter or early spring. An increase in temperature of 4 °C is more likely to influence clearance rates during summer when the rock oysters that dominated the ROB 400s would be closer to their upper thermal limit, especially in scenarios of aerial heatwaves occurring during low tide (Dove and O'Connor 2007; Scanes et al. 2020).

Clearance rates were on average ~45% lower when salinity was ~25 compared to ~15, perhaps because the invertebrates living in the ROB 400s approached their tolerance limit at the lower extremes of salinity these invertebrate communities experience in nature (Oubelkheir et al. 2014; Clementson et al. 2021). Reductions in clearance rates due to exposure to low salinity have been reported for a wide range of invertebrate communities and species (e.g., Navarro and Gonzalez 1998; McFarland et al. 2013; Casas et al. 2018). Our results indicate the invertebrate communities living on the ROB 400s may have been stressed when exposed to very low salinity but seemingly continue to provide substantial filtration services regardless. As clearance rates were greatest at a salinity of ~25, shellfish reef restoration may be as effective in boosting water filtration ecosystem services in estuarine and coastal waterways, where the salinity is usually lower than in the ocean.

Comments and recommendations

The density of microalgae in the control tanks without ROB 400s was not always stable, sometimes declining presumably as microalgae fell to the bottom or slightly increasing as microalgae reproduced or clumps of microalgae broke up. Screening algae cultures prior to use or using a microalga that is neutrally buoyant and/or swims may help to prevent this occurring. The propensity of the density of living microalgae to vary over time highlights the importance of using controls for accurate evaluation of clearance rates. To date, most studies investigating bivalve clearance rates included controls only when performed in a laboratory setting. Filtration studies on invertebrate communities performed by adding microalgae or silt *in situ* often lack controls (e.g., Hansen et al. 2011). We suggest future studies should include controls to avoid overestimating the clearance rates of filter-feeding invertebrate communities.

The ROB 400s used in this study were initially deployed in the intertidal zone and subsequently colonised by organisms adapted to intermittent emersion. This likely explains why these communities appeared to cope well with transport to the laboratory and subsequent experiments. The proportions of deceased shellfish within communities on the ROB 400s at the end of this study are similar to those found on ROB 400s deployed in Moreton Bay (Porter, unpublished data). Likewise, there was only a small spike in ammonia levels in the days after transport, suggesting there was little mortality and decomposition (Canfield et al. 2010). Marine invertebrates living on subtidal modular restoration structures may be less robust when exposed to air compared to the same species living in the intertidal zone (e.g., Widdows and Shick 1985; Giomi et al. 2016). Future studies should consider the need to transport communities in water to reduce lethal and sublethal impacts that could influence clearance rates in subsequent experiments.

This study demonstrates modular restoration structures can be used to test the effects of physiochemical parameters (e.g., light, temperature, salinity) on clearance rates of invertebrate communities. Modular baskets bypass common challenges that occur during filtration studies on bivalves, both *ex situ* (e.g., high level of disturbance, small volumes, often restricted to few animals and/or single species) and *in situ* (e.g., dependence on weather and tide, inability to manipulate physiochemical parameters, lack of controls, poor replication). Additional 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 over the next century (e.g., reduced 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., reduced salinity over weeks).

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Figures

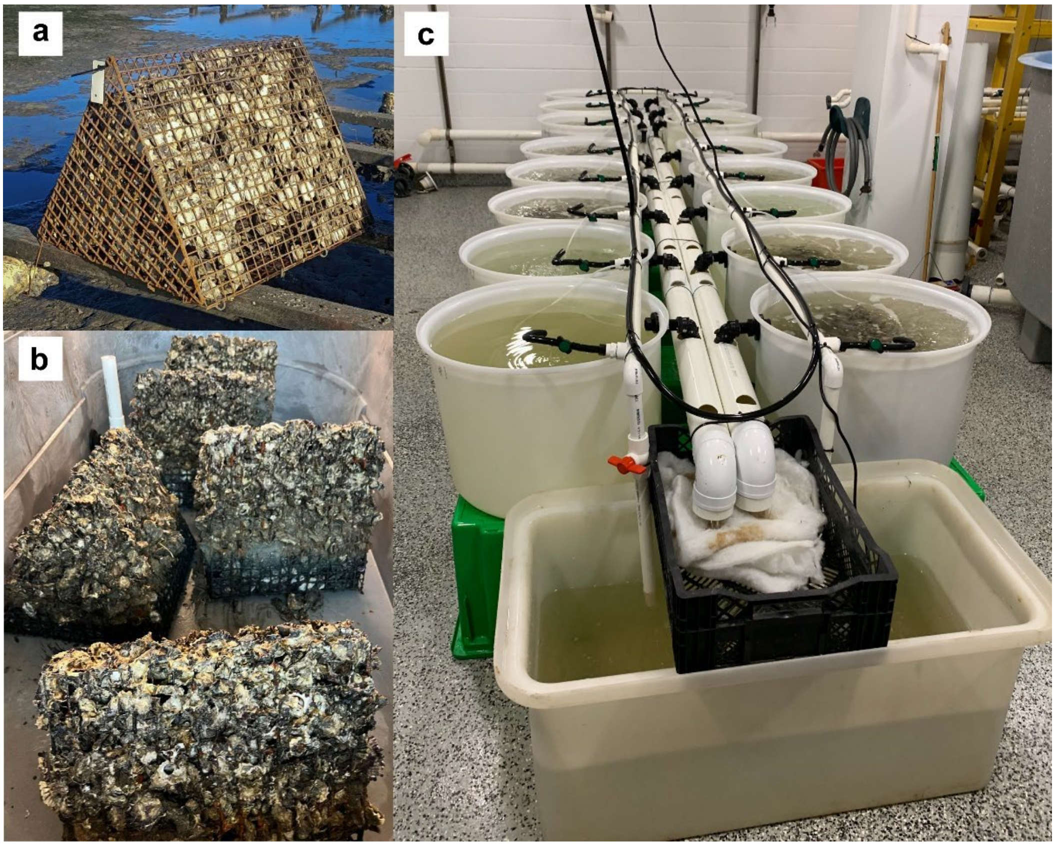

Fig. 1. Modular shellfish reef restoration structures and experimental setup used in this study. **a**. The Robust Oyster Basket (ROB 400) prior to deployment. OzFish Unlimited (n.d.). **b**. ROB 400s colonised by invertebrate communities dominated by rock oysters following 19 months deployment in the intertidal zone in Moreton Bay, Australia. Andersson (2023). **c**. ROB 400s in the experimental setup used to test the effects of light, temperature, and salinity on clearance rates. Mos (2023).

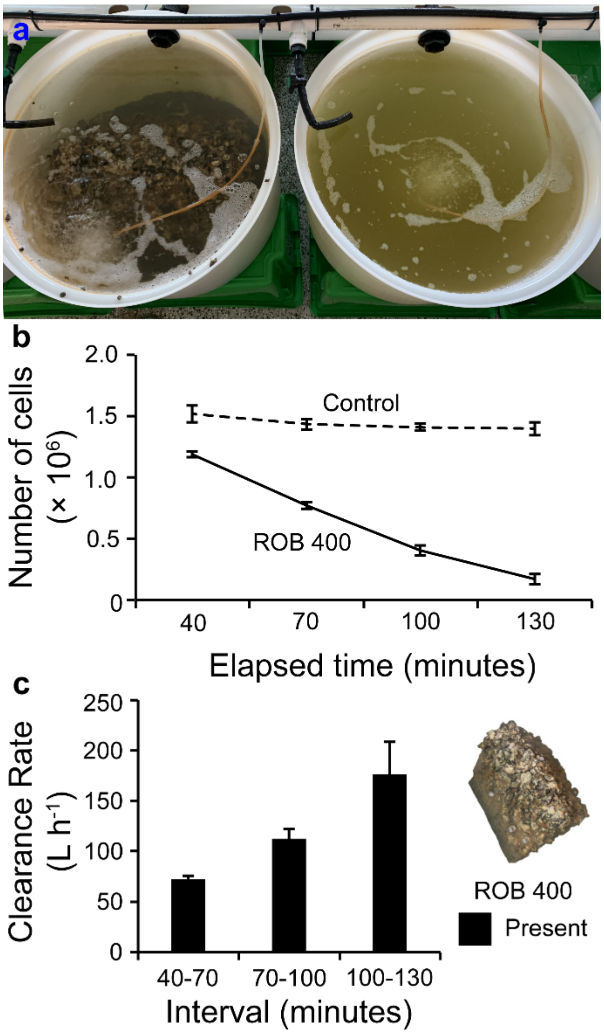

Fig. 2. Clearance of microalgae, *Nannochloropsis oceanica* (CS-246) by marine invertebrate communities living on modular shellfish reef restoration structures (ROB 400). **a**. Illustrative density of *N. oceanica* in tanks with ROB 400s present (left) and absent (right) 2 h after microalgae were added. Mos 2023. **b**. Density of *N. oceanica* in static, aerated tanks without modular shellfish reef restoration structures (Control, dashed line) and with modular shellfish reef restoration structures (ROB 400, solid line). The first measurements were taken 40 min after *N. oceanica* were added to tanks at an initial density of ~1.3 × 10^6 cells mL^{-1}, with subsequent measurements taken every 30 min thereafter. Data are means ± SE, *n* = 9 for ROB 400, *n* = 5 for control. **c**. Clearance rates of invertebrate communities living on ROB 400s in three 30-minute intervals. Data are means ± SE, *n* = 9.

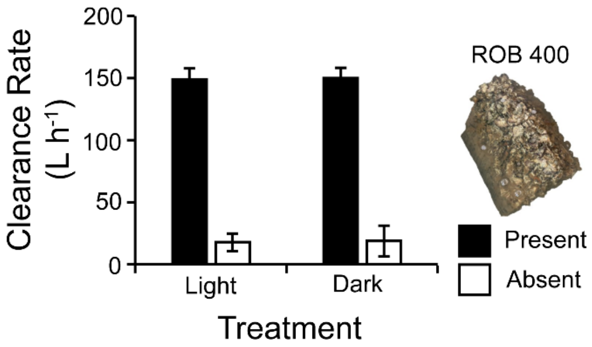

Fig. 3. Effects of light and the presence/absence of modular shellfish reef restoration structures housing rock oyster-dominated invertebrate communities (ROB 400) on clearance rates in static, aerated tanks. Tanks were held in darkness (mean 5.1 lux ± 2.6 SD) or light (mean 455.70 lux ± 5.60 SD) for 18 h before *Nannochloropsis oceanica* CS-246 was added at a density of ~1.1×10^6 cells mL^{-1}. Clearance rates were derived from data on changes in the density of *N. oceanica* after 60 min. Clearance rates were affected by the presence of ROBs (present > absent) 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.

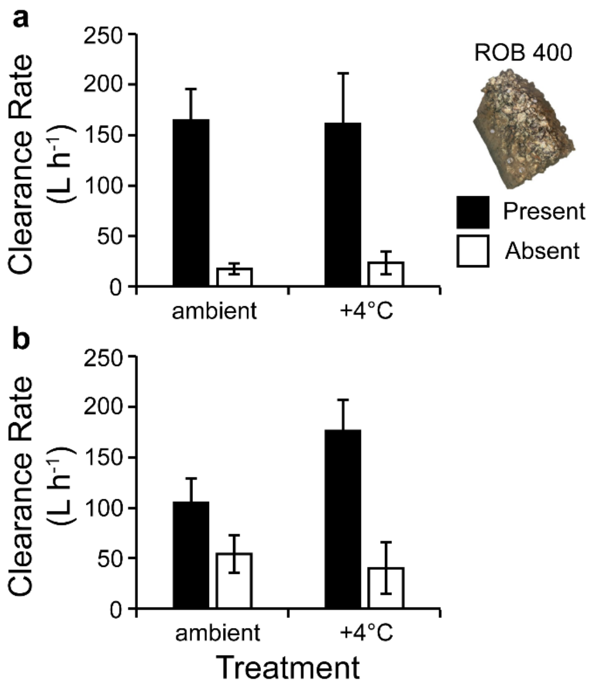

Fig. 4. Effects of temperature and presence/absence of modular reef restoration structures housing rock oyster-dominated invertebrate communities (ROB 400) on clearance rates in static, aerated tanks. Tanks were held at ambient (~21 °C) and warmed (+4 °C, ~25 °C) temperatures for **a**. 24 h and **b**. 120 h before *Nannochloropsis oceanica* CS-246 was added at an initial density of ~1–1.5 × 10^6 cells mL^{-1}. Clearance rates were derived from data on changes in the density of *N. oceanica* after 60 min. Clearance rates were affected by the presence of ROB 400s (present > absent) but were not influenced by temperature, time, nor any interaction among these factors (Table 1). Data are means ± SE; n = 3–5 for present, n = 3 for absent.

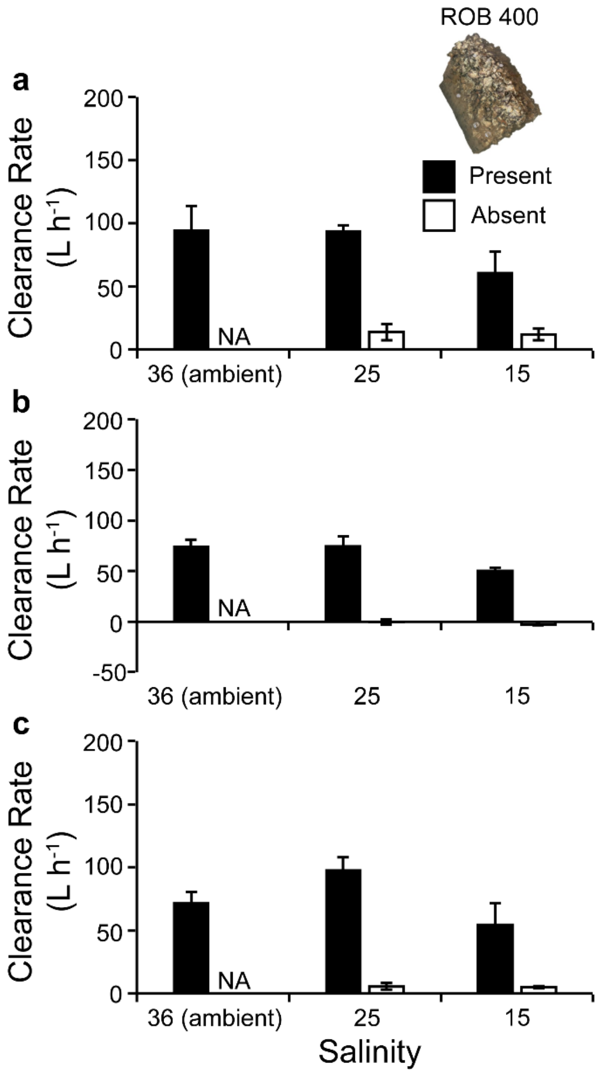

Fig. 5. Effects of salinity and the presence/absence of modular reef restoration structures housing rock oyster-dominated invertebrate communities (ROB 400) on clearance rates in static, aerated tanks. Tanks were held at ambient salinity (~36) or reduced salinity (~25 or ~15) for **a**. 1 day, **b**. 3 days, and **c**. 5 days before *Nannochloropsis oceanica* CS-246 was added at a density of $\sim 1.5 \times 10^6$ cells mL^{-1}. Clearance rates were derived from data on changes in the density of *N. oceanica* after 120 min. No data for ambient salinity-absent treatment was available (NA). Note different scale for the y-axis in panel **b**. Clearance rates for tanks with (present) and without (absent) ROB 400s were statistically analysed separately (Table 1). When ROB 400s were present, clearance rates were affected by salinity (25 = 35 > 35 = 15) but were not influenced by day nor any interaction between these factors (Table 1). When ROB 400s were absent, clearance rates varied across day (day 1 = day 5 > day 5 = day 3) 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 on day 3 and 5 where $n = 4$; $n = 3$ for absent.

Tables

Table 1. Outcomes of PERMANOVA analyses examining the effects of light, temperature, and salinity on the clearance rates of tanks with and without invertebrate communities living on modular shellfish reef restoration structures (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**	**6.45E4**	**185.80**	**.0002**	present > absent
	treatment	1	3.63	0.01	.981	
	p/a × treatment	1	0.03	7.51E-5	.994	
	residual	12	346.99			
Temperature	day	1	602.37	0.26	.627	
	presence/absence	**1**	**1.04E5**	**16.07**	**.0007**	present > absent
	temperature	1	1.24E3	0.22	.662	
	p/a × temp	1	2.03E3	0.34	.577	
	p/a × day	1	3.53E3	1.57	.245	
	temp × day	1	1.74E3	0.75	.408	
	temp × day × p/a	1	6.26E3	3.00	.120	
	replicate (temp × p/a)	12	5.90E3	2.83	.063	
	residual	9	2.09E3			
Salinity (Present)	**treatment**	**2**	**2.67E3**	**4.26**	**.023**	25 = 35 > 35 = 15
	day	2	631.88	1.59	.255	
	treatment × day	4	173.63	0.44	.761	
	replicate (treatment)	7	546.94	1.38	.292	
	residual	14	397.20			
Salinity (Absent)	treatment	1	11.19	0.30	.698	
	day	**2**	**325.66**	**8.05**	**.012**	day 1 = day 5 > day 5 = day 3
	treatment × day	2	0.99	0.02	.978	
	replicate (treatment)	4	37.50	0.93	.501	
	residual	8	40.44			

Supporting Information for

Quantifying clearance rates of restored shellfish reefs using modular baskets

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This file includes:

Tables S1 to S2

Figure S1

Table S1. Mean water parameters (temperature (°C), salinity, and dissolved oxygen (mg L^{-1})) for **a.** temperature and **b.** salinity treatments during laboratory experiments testing clearance rates in tanks containing mixed invertebrate communities living on oyster baskets (ROB 400) and tanks without ROB 400s (Control). Values in parentheses are standard deviation. – data not available

Experiment		**Treatment**	**Elapsed time (h)**	**Temperature**	**Salinity**	**Dissolved oxygen**
a	ROB 400	+4°C	24	24.1 (0.2)	–	–
	Control	+4°C	24	24.7 (0.7)	–	–
	Rob 400	Ambient	24	21.9 (0.1)	–	–
	Control	Ambient	24	21.7 (0.2)	–	–
	ROB 400	+4°C	72	24.1 (0.2)	–	–
	Control	+4°C	72	24.1 (0.8)	–	–
	ROB 400	Ambient	72	19.5 (0.2)	–	–
	Control	Ambient	72	19.4 (0.1)	–	–
	ROB 400	+4°C	120	24.0 (0.2)	39.5 (0.1)	4.8 (0.3)
	Control	+4°C	120	24.3 (0.8)	38.9 (0.2)	4.7 (0.2)
	ROB 400	Ambient	120	19.3 (0.1)	38.3 (0.2)	6.9 (0.5)
	Control	Ambient	120	19.1 (0.3)	37.8 (0.3)	6.9 (0.8)
b	ROB 400	15 salinity	24	21.0 (0.1)	15.5 (0.5)	6.9 (0.2)
	Control	15 salinity	24	21.0 (0.1)	15.1 (0.1)	7.2 (0.3)
	ROB 400	25 salinity	24	20.9 (0.1)	25.6 (0.2)	6.0 (0.2)
	Control	25 salinity	24	21.0 (0.1)	24.7 (0.2)	7.1 (0.1)
	ROB 400	36 salinity	24	21.0 (0.1)	35.7 (0.3)	6.0 (0.1)
	ROB 400	15 salinity	72	21.1 (0.1)	15.8 (0.1)	7.1 (0.4)
	Control	15 salinity	72	20.8 (0.1)	15.7 (0.1)	8.0 (0.7)
	ROB 400	25 salinity	72	21.0 (0.1)	26.0 (0.2)	6.2 (0.3)
	Control	25 salinity	72	21.0 (0.1)	24.8 (0.1)	6.6 (0.4)
	ROB 400	36 salinity	72	21.0 (0.1)	36.4 (0.1)	6.3 (0.3)
	ROB 400	15 salinity	120	21.5 (0.1)	16.7 (0.1)	7.3 (0.2)
	Control	15 salinity	120	21.2 (0.1)	16.1 (0.2)	7.8 (0.2)
	ROB 400	25 salinity	120	21.4 (0.1)	26.1 (0.3)	7.1 (0.3)
	Control	25 salinity	120	21.4 (0.1)	25.3 (0.2)	6.5 (1.1)
	ROB 400	36 salinity	120	21.3 (0.1)	36.8 (0.1)	5.9 (0.2)

Table S2. Information on the tank setup and robust oyster baskets (ROB 400) used in experiments; the volume of seawater in each tank (L), the surface area of each ROB colonised by filter-feeding invertebrates (m^2), the number of living and non-living (in parentheses) individuals (>1 cm) of the dominate filter-feeders found on the ROB 400s, the total number of filter feeders (>1 cm) on each ROB, the height and length (mean ± SD) of 20 rock oysters haphazardly measured from each ROB 400, and the treatment randomly allocated to each tank for the three experiments (light/dark, heatwave, and flood).

Tank #		**Volume of seawater in tank (L)**	**Surface Area m^2**	**rock oysters *Saccostrea* spp.**	**hairy mussel *Trichomya cf. hirsuta***	**pearl oyster *Pinctada* cf. *albina***	**total number of filter feeders**	**alive (%)**	**height mm**	**length mm**	**Light/dark**	**Temperature**	**Salinity**
1	Control	107									Light	Ambient	15
2	Control	107									Dark	Ambient	25
3	ROB 400	81.5	0.246	93 (30)	1 (0)	0 (5)	94 (35)	72.9	26.8 (6.9)	32.7 (8.7)	Dark	Ambient	15
4	ROB 400	80	0.259	102 (55)	3 (0)	8 (5)	113 (60)	65.3	26.5 (5.4)	21.8 (6.0)	Light	Heated	36
5	ROB 400	80.5	0.285	121 (43)	0 (0)	5 (8)	126 (51)	71.2	23.8 (9.2)	32.7 (6.9)	Dark	Heated	36
6	ROB 400	80	0.289	170 (144)	7 (1)	3 (3)	180 (148)	54.9	26.0 (9.0)	21.4 (5.4)	Dark	Ambient	25
7	Control	107									Light	Heated	25
8	ROB 400	80	0.299	122 (65)	65 (0)	18 (4)	205 (69)	74.8	21.8 (6.9)	36.0 (8.60)	Light	Heated	25
9	ROB 400	82	0.264	104 (80)	36 (0)	7 (0)	147 (80)	64.8	29.9 (9.6)	21.7 (7.6)	Dark	Ambient	25
10	Control	107									Dark	Heated	15
11	Control	107									Light	Ambient	15
12	ROB 400	83	0.315	157 (138)	25 (1)	8 (7)	190 (146)	56.5	24.5 (6.1)	32.9 (3.2)	Light	Heated	36
13	ROB 400	85.5	0.310	84 (53)	2 (0)	3 (3)	89 (56)	61.4	29.2 (8.4)	25.6 (9.5)	Light	Ambient	25
14	Control	107									Dark	Heated	25
15	ROB 400	81	0.309	169 (173)	22 (0)	2 (10)	193 (183)	51.5	26.6 (7.4)	32.0 (6.6)	Dark	Heated	15
16	ROB 400	81	0.298	172 (103)	7 (1)	0 (4)	179 (108)	62.4	31.5 (9.5)	25.4 (8.3)	Light	Ambient	15
		MEAN	0.287	129.4	16.8	5.4	151.6	63.6					
		±SE	0.024	10.9	6.6	1.7	43.5	2.5					

The mean total surface area colonised by invertebrates on each ROB did not differ among treatments for the light/dark (ANOVA, $F_{1,8} = 1.44$, $p = .242$), temperature ($F_{1,8} = 0.61$, $p = .458$), or salinity ($F_{2,7} = 0.05$, $p = .925$) experiments.

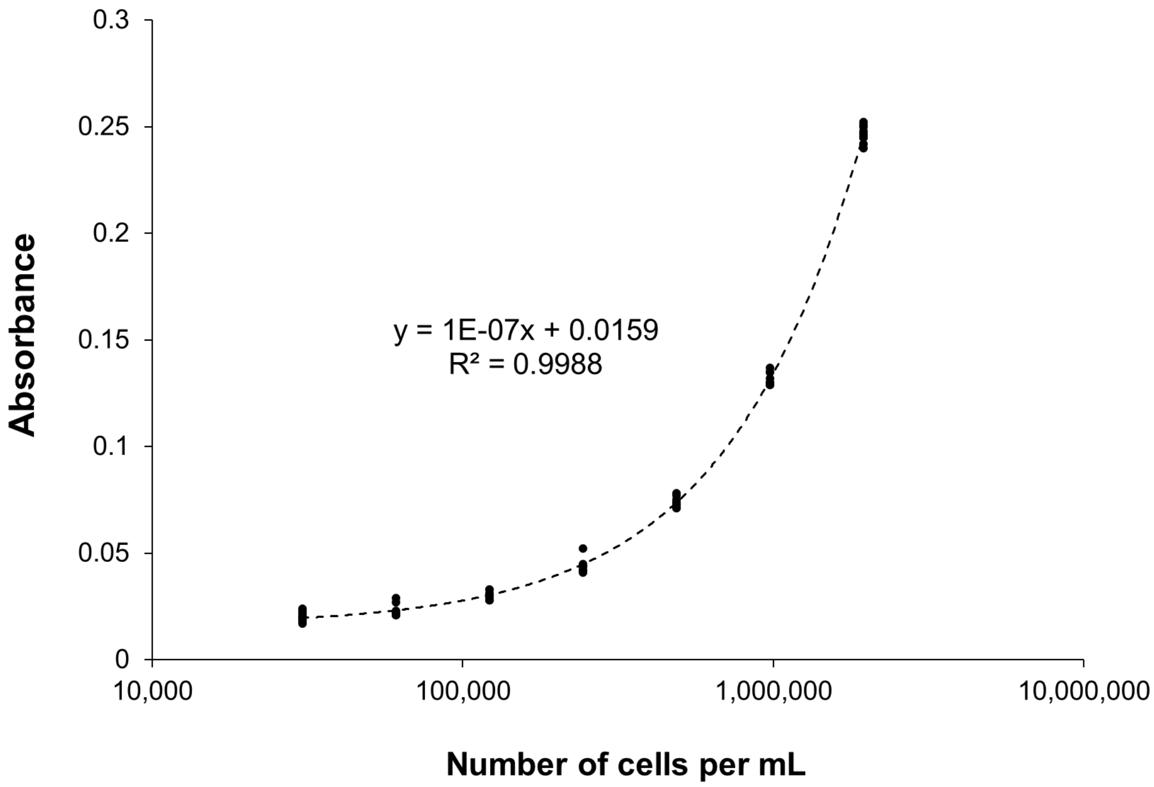

Fig. S1. Relationship between the number of cells per mL of *Nannochloropsis oceanica* (log scale) and total absorbance (sum of λ = 750, 664, 647, 630 nm) measured using a spectrophotometer (Hach DR 5000™ UV-Vis, Starn Pty Ltd glass cuvette, Type 1, match code 7, path length 10 mm).