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Filtration rate and bioremediatory potential of the tropical blacklip rock oyster *Saccostrea* lineage J

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ABSTRACT: The tropical blacklip rock oyster *Saccostrea* lineage J is an emerging aquaculture species displaying fast growth rates, large sizes and resilience to fluctuations in temperature and salinity, all characteristics that suggest it would be well-suited to bioremediatory applications. To investigate their bioremediatory potential, the present study aimed to (1) determine the influence of temperature (20, 24, 28, 32°C) on the filtration rate of *Saccostrea* lineage J and (2) describe and quantify uptake in total nitrogen (TN), total phosphorus (TP), total suspended solids (TSS) and chlorophyll *a* (chl *a*), using prawn pond effluent and 2 levels of oyster stocking density. The results demonstrated that higher water temperatures promote a faster filtration rate and identified an optimal performance range of 24 to 32°C for a filtration rate of 12.68 to 15.20 l h^{-1} g^{-1}. In addition, the highest density (0.66 oysters l^{-1}) of stocked oysters resulted in significant reduction of all water quality parameters, with TN reduced by 13%, TP by 16%, TSS by 95% and chl *a* by 29% when compared to unstocked controls after 3 h. Tissue analysis of 10 oysters with a mean whole weight of 75.4 g revealed a mean of 0.09 g of nitrogen per oyster. Scaling these values suggests that 1.20 kg of nitrogen is removed per tonne of harvested oysters. This study is the first to investigate the bioremediatory potential of *Saccostrea* lineage J and demonstrates their potential to improve aquaculture wastewater treatment practices and bioremediation.

KEY WORDS: Bioremediation · Aquaculture · Wastewater · Reef credit · Nutrient credit trading · Nitrogen · Phosphorus · Total suspended solids

1. INTRODUCTION

The global aquaculture industry is one of the fastest growing primary production sectors, with an average annual growth rate of 6.7% between 1990 and 2020 (FAO 2022). There is an increasing desire to expand and intensify land-based production facilities culturing species such as prawns, which require a large volume of water for growout phases (Webb et al. 2012). In many countries, intensive aquaculture facilities take in water from natural environments and then discharge culture water back into these environments via settlement ponds (Verdegem 2013). Significant planning and water flow testing is required to select suitable production areas with sufficient tidal currents to effectively disperse effluent (Nasir et al. 2015). If effluent is not dispersed successfully, the nutrient-rich wastewater (e.g. high in nitrogen and phosphorus) can degrade marine environments through eutrophication and algal blooms and by

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smothering benthic environments (Ziemann et al. 1992). As the environmental sustainability of the global aquaculture industry draws increased attention, such negative ecological impacts are impacting its social license (Billing 2018).

The Great Barrier Reef Marine Park (GBRMP) is adjacent to the Queensland (Australia) coastline and includes numerous key catchment areas. Despite the relatively small amount of nutrients discharged from the aquaculture sector in comparison to other industries, such as sugarcane farming (Jegatheesan et al. 2007), the rapid growth of the aquaculture industry demands research on the reuse and treatment of farm wastewater entering the GBRMP. All land-based farms in Australia are required to follow discharge standards that strictly limit the release of culture effluent back into the environment (Brennan 2002). These discharge restrictions can ultimately limit the stocking density, overall capacity and future expansion potential of land-based aquaculture facilities (Brennan 2002). However, due to the small size of suspended organic and inorganic matter within effluent water, mechanical filtration processes are often difficult to achieve and expensive (Hopkins et al. 1995).

The ecological concerns surrounding effluent discharge, in addition to costly water treatment practices, have prompted interest into biological filtration methods. One possibility entails using marine bivalves to remove particulate matter from suspension and decrease nutrient levels (Shpigel & Blaylock 1991, Shpigel et al. 1993). Bioremediation refers to the process of removing contaminants through biological means (Peña-Messina et al. 2009). Bivalves are a preferred bioremediatory group due to their tolerance of a wide range of environmental conditions and resilience to poor water quality (Martinez-Cordova & Martinez-Porchas 2006). The organic content of wastewater from aquaculture environments (e.g. faecal matter and undigested food) can provide a rich supply of food for bivalves such as oysters; in addition, they can assist in the removal of fine sediments from the water column. This is because fine inorganic particles are ingested along with food during the natural feeding process of oysters. These particles are then coagulated into larger, heavier particles and excreted as pseudofaeces (Tenore & Dunstan 1973). Previous studies investigating the positive effects of oyster filtration have demonstrated the ability of the Sydney rock oyster *Saccostrea glomerata* to significantly reduce the concentration of nitrogen (N), phosphorus (P), bacteria, phytoplankton and other particulate matter within prawn culture effluent (Jones & Preston 1999). In addition to the ecological benefits and reduced strain on mechanical filtration methods, the recovery of nutrients from uneaten pellet feeds and prawn waste could provide economic benefit through production of a second cash crop. Oysters used for bioremediation would need to be tested to ensure that they comply with the Food Standards Australia New Zealand Food Standards Code and Export Orders for bivalve molluscs.

The effects of environmental factors such as temperature, salinity and food availability on temperate oyster (Bayne et al. 1999, Calvo et al. 2001) and tropical oyster (Enríquez-Ocaña et al. 2012, Guzmán-Agüero et al. 2013) feeding patterns have been extensively reviewed in the literature. However, as an emerging tropical aquaculture species, the feeding patterns of adult *Saccostrea* lineage J remain unstudied. Temperature-reliant filtration rate is a key factor that governs the aerobic capacity of oysters (Eymann et al. 2020). Filtration is fundamental for both oxygen uptake and nutrient retention, and therefore influences the organism's metabolic capacity, energy budget and subsequent growth capacity (Kittner & Riisgård 2005). Quantifying the amount of suspended solids or nutrients removed from the water column via filtration enables calculation of the overall ecosystem service of both oyster farms and bioremediation programs. The quantification of filtration rate has prompted some controversy and debate resulting from variation in methodology, which makes it difficult to make direct comparisons between species (Peña-Messina et al. 2009). Several studies investigating oyster feeding behaviour have described a pattern of steady filtration rate increase as water temperatures increase, provided the animals were within their identified thermal tolerance window (Enríquez-Ocaña et al. 2012, Guzmán-Agüero et al. 2013, Eymann et al. 2020). This trend is typically followed by an inflexion point indicating maximal filtration rate, after which the water warms beyond an optimal temperature. As *Saccostrea* lineage J is a species with high thermal tolerance, it may be capable of reaching greater filtration rates than temperate species.

Multiple studies have investigated the bioremediatory potential of bivalves, using a variety of methods and applications. Filtration rate is a key component of evaluating bioremediatory potential and is typically quantified by the collection of biodeposits (Tenore & Dunstan 1973, Hoellein et al. 2015) or measuring the reduction in algae cell density over time (Bayne et al. 1999, Guzmán-Agüero et al. 2013). Studies investigating the net uptake and removal of nutrients and pollutants by bivalve farms undertake tissue analyses to determine nutrient and heavy metal concentrations

and estimate their removal through harvest. Trials assessing the overall ecosystem services of bivalves tend to analyse the deposition rate and subsequent impact on the substrate (Beseres Pollack et al. 2013). When assessing the applied use of bivalves in a commercial aquaculture setting, the most common methodology involves culture effluent being pumped through a series of aquaria containing shellfish. Water samples are collected before and after bivalve filtration and key water quality parameters are measured (Jones et al. 2001, 2002).

The tropical blacklip rock oyster *Saccostrea* lineage J is an emerging aquaculture species displaying several characteristics that suggest it would be well-suited to bioremediatory applications. These include a large size and fast growth as well as resilience to fluctuations in temperature and salinity (Nowland et al. 2019a,b). This species displays a wide geographic distribution throughout the tropics (Lindsay 1994, Nowland et al. 2019c). Within Australia, it has been reported from waters as far south as Bowen on the east coast of Queensland, as far north into Cape York and the Northern Territory and as far west as Cone Bay in Western Australia (Nowland et al. 2019c). This naturally occurring range coincides with many successful prawn farming regions, and suggests this species would be compatible with seasonal variation at these locations.

The aims of the present study were to (1) evaluate the effect of temperature on *Saccostrea* lineage J filtration rate and (2) describe and quantify the uptake in total nitrogen (TN), total phosphorus (TP), total suspended solids (TSS) and chlorophyll *a* (chl *a*) from prawn pond effluent exposed to 2 levels of oyster stocking density. The results of this study will quantify the filtration rate of tropical blacklip rock oysters at a range of temperatures and will enable evaluation of their potential for bioremediation of prawn aquaculture effluent.

2. MATERIALS AND METHODS

2.1. Assessing filtration rate

2.1.1. Experimental design

Market size (>70 mm) tropical blacklip rock oysters *Saccostrea* lineage J (n = 70) were purchased from Bowen Fresh Oysters. During acclimation, the oysters were divided among 4 separate recirculating holding systems, each comprising one 290 l sump and six 35 l aquaria. During acclimation, the oysters were fed at a standard rate of 2 billion cells per oyster per day. The tanks were heated using element heaters (Scintex) and the oysters were acclimated from the ambient farm temperature of 28°C at a rate of 2°C d^{-1} until their treatment temperature was reached . The 4 treatment temperatures were 20, 24, 28 and 32°C. These temperatures span the range of water temperatures recorded across the species' known range in Australia (Cobcroft & Jerry 2020). The experiment did not aim to determine the thermal threshold of *Saccostrea* lineage J.

2.1.2. System design

Using a static design, 18 aquaria were each filled with 15 l of 0.2 µm filtered seawater at 36 ppt and salinity was maintained throughout the experiment. The specific trial temperature was maintained by placing 6 aquaria within 3 respective 290 l holding tanks containing an element heater and a 240 V submersible pump (Aqua-nova) to ensure water temperature was evenly distributed. Room temperature was maintained by setting the air conditioning to 2°C below the desired treatment temperature the day prior to the experiment, to cool treatment water and ensure temperatures below ambient could be successfully maintained. Oysters were starved for 24 h before the filtration rate trial commenced. Each aquaria contained a single oyster and known quantity of algae, 375 000 cells ml^{-1} of *Chaetoceros muelleri* (Gray & Langdon 2018). Aeration stones were added to each aquaria to keep algae cells in suspension. Each of the 3 sumps contained 1 control aquaria, without oysters, which received the same quantity of algae.

2.1.3. Sampling and biological data

Experiments for each temperature treatment were run simultaneously. Oysters were allowed to filter for a period of 5 h. Three replicate 1.4 ml samples of water were taken from each aquaria at the commencement of the trial and at the end of each hour (1, 2, 3, 4 and 5). Samples were fixed using 0.1 ml of 10% seawater buffered formalin to preserve algal cells and allow the concentration of cells to be determined later using a microscope and hemocytometer. The temperature of each individual tank was recorded hourly using a handheld thermometer (Soffritto).

At the completion of the trial period, all bivalves were measured for shell length (dorso-ventral measurement; DVM), width (antero-posterior measure-

ment; APM) and depth. Whole oyster weight was recorded, then tissue was removed and wet weight was recorded to a precision of 0.01 g. Using a pre-weighed and dried paper liner, oyster tissues were dried using a drying oven at 70°C for 24 h to achieve a constant dry weight. Dried tissues were weighed using scales accurate to 0.01 g.

2.1.4. Data analysis

All data were assessed for normality and homogeneity of variance using qq plots; data for oyster dry weights required log transformation. To determine the influence of temperature on oyster filtration rate over time, a mixed effects model was used, with hour, treatment and their interaction as fixed effects and sump and aquaria as random effects. Two alternative models were tested, one with hour as a discrete variable and another with hour as a continuous variable with quadratic curve. The model with hour as continuous variable was chosen as it had a lower Akaike information criterion (AIC). Significance of modelled factors was tested using Wald chi-squared tests and models were fitted using the lme4 package (Bates et al. 2015) in R. Filtration rate was obtained using the indirect method of measuring decrease in algal cell concentration over time. The filtration rate formula (Coughlan 1969) describes the hourly volume of water filtered by an oyster, per gram of dry tissue weight:

$$FR = (V/nt)\ln(C0/Ct)/w \quad (1)$$

where V = volume of water per aquarium (ml), n = number of animals per aquarium, t = time (h), C0 and Ct = algal concentrations at time t−1 and time t (h), respectively, and w = dry oyster tissue weight (g).

2.2. Bioremediation of prawn culture water

2.2.1. Experimental design

An additional 100 market size (>70 mm) oysters were sourced from Bowen Fresh Oysters. From these oysters, 10 were chosen at random to be dissected and analysed for total tissue nitrogen. The remaining 90 were acclimated to 28°C at a rate of 0.5°C d^{-1} over a period of 2 d. Salinity and pH were measured daily (see Tables A1 & A2 in the Appendix). During acclimation, the oysters were fed at a standard rate of 2 billion cells per oyster per day. Oysters were starved for 24 h before the filtration rate trial commenced. Using a static system, a total of 9 rectangular aquaria were filled to 30 l from a sump containing prawn culture effluent, obtained from Pacific Reef prawn farm, Ayr, Queensland, Australia, 12 h before the experiment commenced. The water temperature was 31°C and salinity was 35 ppt at the time of collection.

Three treatments were compared: high density, containing 20 oysters (0.66 oysters l^{-1}), medium density, containing 10 oysters (0.33 oysters l^{-1}) and control, containing no oysters. Each treatment contained 3 replicate aquaria. Water temperature was controlled via air conditioning and was set to 28°C to reflect the yearly average farm water temperature. Oysters were suspended on layered mesh in the water column of the tanks, approximately 15 cm above the bottom, to allow water circulation.

2.2.2. Sampling and biological data

Taking care not to disturb feeding oysters, initial water samples were collected, including 500 ml for analysis of TSS, 500 ml for analysis of chl *a* and 60 ml for analysis of both TN and TP. Three replicate water samples per parameter were collected from the sump prior to pumping into the aquaria. After placement in aquaria, oysters were allowed 10 min to resume their natural feeding pattern before the 3 h filtration trial period commenced. Samples for TSS, chl *a*, TN and TP were collected at the completion of each hour (1, 2 and 3). At the completion of the experiment, all oysters were measured for shell length, width and depth to the nearest mm and whole oyster weight to 0.1 g.

2.2.3. Total suspended solids (TSS)

To determine the total mass of suspended solids, each sample was passed through a pre-dried and weighed Whatman glass microfiber filter, using a buccal flask and funnel assembly. Filters were dried for a period of 24 h at 104°C to ensure they achieved a constant weight and reweighed using a scale accurate to 0.0001 g. The overall mass of TSS was determined by comparing the initial and final weights.

2.2.4. Total nitrogen (TN) and total phosphorus (TP)

TN and TP samples were analysed at the Centre for Tropical Water & Aquatic Ecosystem Research (TropWATER) laboratory at James Cook University, Townsville, Australia following standard methods for the examination of water and wastewater (American Public

Health Association 2005). Analysis for total nitrogen and total phosphorus was carried out using the alkaline persulfate digestion of unfiltered water samples (Hosomi & Sudo 1986). Samples were digested in an autoclave for 70 min at 121°C with an alkaline persulfate digest reagent. Values for TN and TP were then simultaneously determined by APHA 4500-NO_3^- F and APHA 4500-P F colorimetric methods on an OI Analytical F3700 Segmented Flow Analyser.

2.2.5. Chlorophyll *a*

Chl *a* samples were also analysed by the TropWATER laboratory. To determine the concentration of chl *a*, samples were first filtered through glass fiber filters. Each sample was placed in a tissue grinder with 2–3 ml of 90% aqueous acetone solution and macerated at 500 rpm for 1 min. Each sample was then transferred to a screw-cap centrifuge tube and the total volume adjusted to 10 ml with 90% aqueous acetone. Samples were allowed to steep for 2 h at 4°C in the dark. Samples were then clarified by filtering through a solvent-resistant disposable filter. Following this, 3 ml of clarified extract were transferred to a 1 cm cuvette and the optical density (OD) was measured at 750 and 664 nm using a spectrophotometer. The extract was then acidified in the cuvette with 0.1 ml 0.1 M HCl, before being agitated and read at OD 750 and 665 nm 90 s after acidification. From these values, the concentrations of chl *a* per m^3 were calculated.

2.2.6. Total tissue nitrogen

Oyster tissue samples were analysed by the Environmental Analysis Laboratory, Southern Cross University, Lismore, NSW, Australia. Whole weights were recorded to 0.01 g, before the oysters were sacrificed and tissue was removed from the shells. Wet tissue weight was also recorded to 0.01 g. Tissue samples were frozen at −25°C prior to transport. Samples were defrosted and dried at 40°C for 48 h prior to crushing and analysis. Total nitrogen analysis followed the methods described by Rayment & Lyons (2012).

2.2.7. Data analysis

All data were tested using qq plots to assess normality and homogeneity of variance. Whole oyster weight was log transformed to meet assumptions of normality. To analyse the effect of oyster density on water quality parameters (TN, TP, TSS and chl *a*), a mixed effect model was used with hour, treatment and their interaction as fixed effects and aquaria and replicate samples as random effects. As in the filtration rate experiment, we tested alternative models with hour as a continuous or discrete variable. In this case the model with hour as a discrete variable was preferred, as it had a lower AIC. Statistical significance of modelled parameters was tested using Wald chi-squared tests. Mixed effects models were fitted using the lme4 package (Bates et al. 2015) and all analyses were completed and plots created using R (R Core Team 2019).

3. RESULTS

3.1. Assessing filtration rate

3.1.1. Biometric data and filtration rate

No significant differences in mean oyster whole weights, dry tissue weight, DVM or APM were recorded between the 4 temperature treatments (Table A3 in the Appendix). All 4 treatments reached the desired temperature (±1°C) and experienced minimal variation (≤0.5°C) throughout the 5 h trial period (Table A3).

Oyster filtration rate differed significantly between temperature treatments ($\chi^2 = 2582$, df = 3, $p < 0.001$) and over time ($\chi^2 = 2607$, df = 2, $p < 0.001$); the interaction of treatment and time was also significant ($\chi^2 = 2338$, df = 6, $p < 0.001$). Oysters within the 32°C treatment achieved the fastest filtration rate within the first hour, reaching 3.54 l h^{-1} g^{-1}, which steadily increased until hour 3 before plateauing in hour 4 at a maximum filtration rate of 14.67 l h^{-1} g^{-1}. The oysters within the 24 and 28°C treatments both displayed a similar pattern of filtration rate increasing with time, completing the 5 h trial period without plateauing at rates of 15.20 and 12.68 l h^{-1} g^{-1} respectively. The oysters in the 20°C treatment demonstrated a more gradual increase in filtration rate, which remained lower than the 3 warmer treatments, reaching a maximum rate of 4.89 l h^{-1} g^{-1} in hour 4 (Fig. 1).

3.2. Evaluating bioremediation potential

3.2.1. Biometric data

No significant difference in oyster mean whole weights, DVM or APM was detected between tanks or treatments (Table 1).

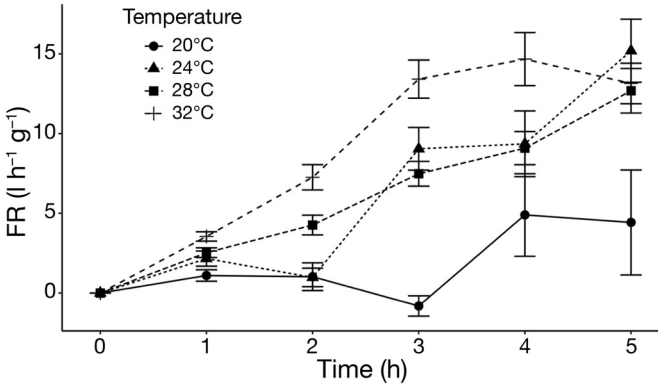

Fig. 1. Filtration rate (FR, l h^{-1} g^{-1}) of tropical blacklip oysters *Saccostrea* lineage J over a 5 h trial period at 4 temperatures (20, 24, 28 and 32°C). Error bars indicate SE

Table 1. Mean whole weight, shell dorso-ventral measurement (DVM), antero-posterior measurement (APM) of oysters in 2 density treatments. Each density treatment was replicated 3 times

Density	Mean whole weight (g) ± SE	Mean DVM (mm) ± SE	Mean APM (mm) ± SE
Medium	82.77 ± 4.49	72.0 ± 1.5	58.8 ± 2.2
Medium	74.78 ± 3.21	67.6 ± 1.5	54.2 ± 1.7
Medium	81.66 ± 2.13	71.3 ± 0.9	56.0 ± 1.8
High	75.01 ± 2.67	70.6 ± 1.0	53.5 ± 1.6
High	82.62 ± 3.60	71.7 ± 1.7	55.3 ± 1.3
High	80.32 ± 2.74	72.3 ± 1.2	53.5 ± 1.4

3.2.2. Bioremediation

Filtration by oysters removed a significant quantity of suspended organic matter from the water column, and the amount removed tended to be larger at higher stocking densities. The high density of stocked oysters resulted in TN being reduced by 21%, TP by 27%, chl *a* by 39% and TSS by 99% (Fig. 2, Table 2). In the control treatment, containing no oysters, most of the particulate matter remained suspended in the water column for the 3 h duration of the experiment. Within the control treatment, TN was reduced by 9%, TP by 13%, chl *a* by 14% and TSS by 71% (Fig. 2). The bioremediation effect of oysters, relative to controls, was high, with reductions of 13% for TN, 16% for TP, 95% for TSS and 29% for chl *a* at the highest oyster density, at the final timepoint.

TN did not differ significantly with treatment (χ^2 = 2.61, df = 2, p = 0.27), but did vary with time (χ^2 = 130.9, df = 3, p < 0.001) and the interaction of treatment and time (χ^2 = 32/7, df = 6, p < 0.001). Total nitrogen did not decrease in either medium or high-density oyster treatments in the first hour but then

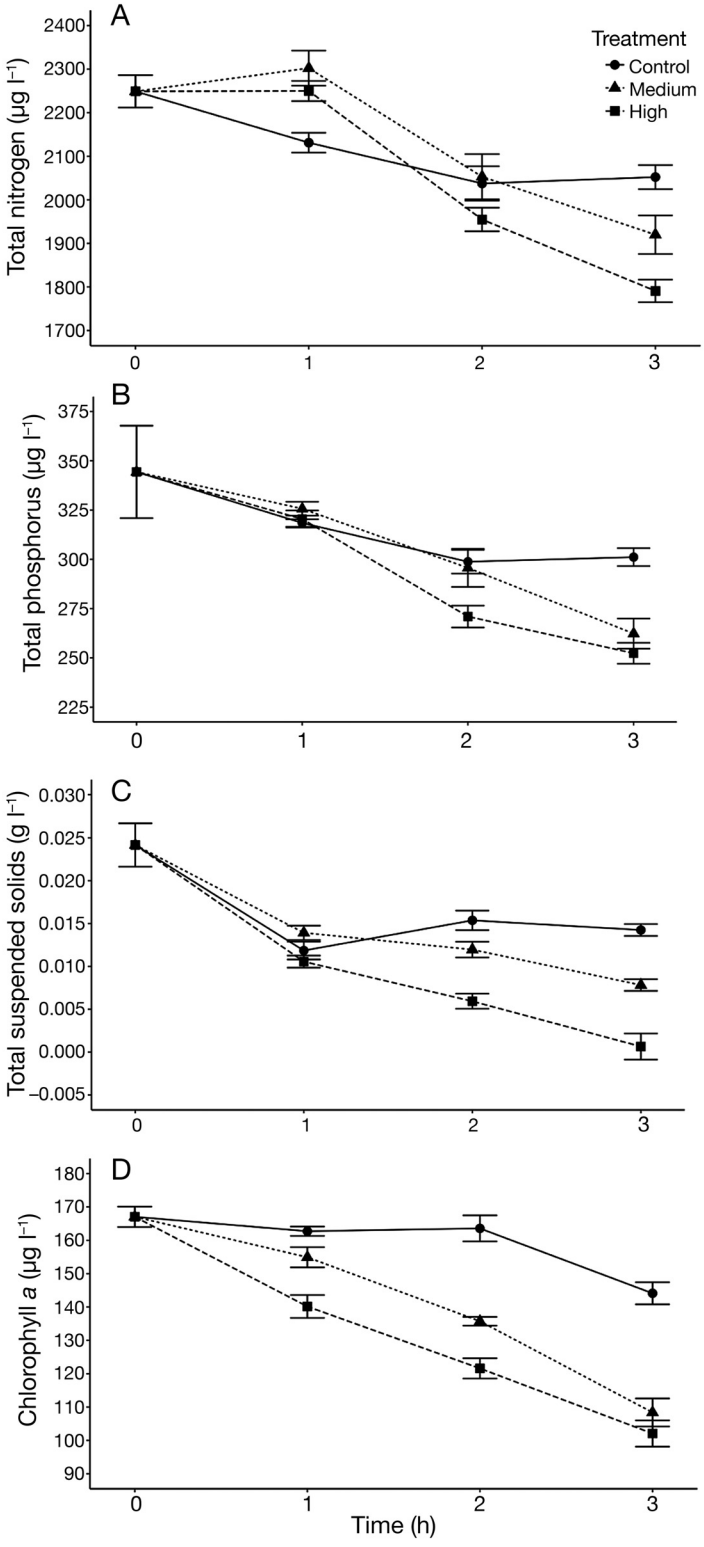

Fig. 2. Changes in (A) total nitrogen (TN, µg l^{-1}), (B) total phosphorus (TP, µg l^{-1}), (C) total suspended solids (TSS, g l^{-1}) and (D) chlorophyll *a* (chl *a*, µg l^{-1}) of control (0 oysters), medium (10 oysters) and high (20 oysters) treatments over the 3 h trial period. Error bars: SE

Table 2. Comparison of Sydney rock oyster and tropical blacklip rock oyster bioremediation studies, detailing system type, filtration time, stocking density and percentage reduction of water quality parameters, including total nitrogen (TN), total phosphorus (TP), total suspended solids (TSS) and chlorophyll *a* (chl *a*)

Species	System	Time (h)	Stocking density (oysters l^{-1})	Raw effluent levels				% reduction				Reference
				TN ($\mu g\ l^{-1}$)	TP ($mg\ l^{-1}$)	TSS ($g\ l^{-1}$)	Chl *a* ($ug\ l^{-1}$)	TN	TP	TSS	Chl *a*	
Sydney rock oyster (*Saccostrea glomerata*)	Static	2	0.70	1400	150	0.13	44.1	20	33	51	92	Jones & Preston (1999)
Blacklip rock oyster (*Saccostrea* lineage J)	Static	3	0.66	2250	340	0.025	165	21	27	99	39	Present study
Sydney rock oyster (*Saccostrea glomerata*)	Flow-through	2	0.72					34	44	71	61	Jones et al. (2002)
Sydney rock oyster (*Saccostrea glomerata*)	Recirculation	2	0.1					NA	NA	84	96	Jones et al. (2002)

decreased rapidly in absolute terms and relative to controls in hours 2 and 3 (Fig. 2A).

The change in TP did not differ significantly between treatments (χ^2 = 8.98, df = 2, p = 0.11); however, it did differ significantly with time (χ^2 = 128.33, df = 3, p < 0.001) and with the interaction of treatment and time (χ^2 = 22.64, df = 6, p < 0.001). Total phosphorus decreased gradually across all 3 treatments within the first hour. In the final 2 h, both the medium and high-density treatments displayed accelerated rates of phosphorus removal from the water column (Fig. 2B).

TSS differed significantly between treatments (χ^2 = 48.94, df = 2, p < 0.001), with time (χ^2 = 151.6, df = 3, p < 0.001) and with the interaction of treatment and time (χ^2 = 42.7, df = 6, p < 0.001). For the final 2 h, the control treatment displayed little to no change. Both the medium and high-density treatments displayed notable reductions in TSS throughout hours 2 and 3, with the high-density treatment concluding the trial period with the lowest TSS (Fig. 2C).

Total chl *a* differed significantly between treatments (χ^2 = 85.7, df = 2, p < 0.001), with time (χ^2 = 157.8, df = 3, p < 0.001) and with the interaction of treatment and time (χ^2 = 25.59, df = 6, p < 0.001) (Fig. 2D). The control treatment remained relatively constant for the first 2 h before declining in the final hour. In the first hour, the high-density treatment exhibited the most rapid reduction in total chl *a*. For the final 2 h, the medium and high treatments both displayed comparable trends in reducing total chl *a*.

There are several common trends among the water quality parameters. All treatments experienced a reduction in all 4 parameters following the 3 h experimental period. The high-density oyster treatment produced the fastest rate of reduction for all water quality parameters. In accordance, at the conclusion of the trial, the lowest concentrations of nitrogen, phosphorus, TSS and chl *a* occurred in the high-density treatment.

Total nitrogen percentage by dry weight of the 10 oysters sampled revealed a range from 6.66 to 9.50% and a mean value of 8.27% (Table 3). At a mean dry tissue weight of 1.08 g, this equates to a mass of 0.09 g of nitrogen removed per oyster. When scaled, this equates to approximately 1.2 kg of nitrogen removed per harvest tonne of oysters.

4. DISCUSSION

The findings highlight that the filtration rate of the tropical blacklip rock oyster *Saccostrea* lineage J is

Table 3. Nitrogen percentage by dry weight of 10 sampled tropical blacklip oysters

Sample number	Whole oyster weight (g)	% N in dry tissue
1	65.22	9.50
2	61.02	9.07
3	82.03	6.95
4	87.64	8.10
5	74.15	9.24
6	79.29	6.66
7	70.47	8.91
8	74.92	7.57
9	76.98	8.64
10	82.53	8.06

closely linked to temperature, corroborating the outcomes of studies on the European flat oyster *Ostrea edulis* (Eymann et al. 2020) and the mangrove oyster *Crassostrea corteziensis* (Enríquez-Ocaña et al. 2012, Guzmán-Agüero et al. 2013). The filtration rates of the 24 and 28°C treatments displayed comparable patterns over time and neither reached a point of inflection. While the 32°C treatment returned the highest filtration rate for much of the 5 h period, it eventually reached an inflection point and plateaued. The inflection point may indicate that the food source available within the 32°C treatment had diminished, therefore causing the filtration rate to peak. Food availability is an important factor influencing filtration rate and should be considered when estimating oyster performance in a specific location (Guzmán-Agüero et al. 2013). The oysters within the 20°C treatment appeared to display an inhibited filtration rate, most likely due to reduced metabolic rate.

The filtration rate of the tropical blacklip rock oyster was between 3 to 5 times that of other frequently cultured oyster species (Table 4). The influence of temperature on the filtration rate of bivalves has been attributed to several physiological mechanisms (Enríquez-Ocaña et al. 2012). One explanation suggests that as water temperature increases, water viscosity decreases, which assists with improved filtration capacity (Specht & Fuchs 2018). Elevated temperatures induce higher metabolic rates but also result in less dissolved oxygen in water, resulting in bivalves pumping harder to compensate (Specht & Fuchs 2018). An alternate theory is that variation in filtration rate is due to increased chemical reactions during metabolism at higher temperatures (Yukihira et al. 2000). There is also likely an interaction between temperature and food availability, which both display large seasonal variations. Food availability typically peaks during periods of high water temperature in northern Australia (Munksgaard et al. 2017). These higher filtration rates relative to those of other species suggest that *Saccostrea* lineage J may be well suited to biofiltration roles.

The results of this study suggest that the tropical blacklip rock oyster has a tolerance to high water temperatures. In accordance with the findings reported by Cáceres-Puig et al. (2007) for the tropical oyster species *C. corteziensis, Saccostrea* lineage J displays a similar thermal tolerance for temperatures above 32°C. Future studies investigating the maximal filtration rate of *Saccostrea* lineage J would benefit from including temperatures higher than 32°C, to find the temperature at which filtration rate is negatively affected. It has been shown that the Sydney rock oyster *S. glomerata* does not reach a metabolic inflection point at 33°C (Parker et al. 2017). This represents an important study topic as it may assist in future selection of sites for its culture and identify locations that are not suitable.

The water quality parameters of concern in prawn farm effluent undergo significant variation as the number of ponds and their stocking density vary with seasonal demand (Brennan 2002) (Table 5). Typically, Australian farms have the highest stocking densities from October to February, as prawns are in peak demand during the summer months. This bioremediation trial was conducted during the month of May; however, TN, TP, TSS and chl *a* were higher than in May of previous years. The level of chl *a* in the effluent was indicative of high phytoplankton productivity within the culture ponds.

Table 4. Comparison of filtration rates (FR) of 4 oyster species. Temperatures at which observations were made are listed

Species	Water temperature (°C)	FR ($l\ h^{-1}\ g^{-1}$)	Reference
Sydney rock oyster (*Saccostrea glomerata*)	20	3.10	Bayne et al. (1999)
European flat oyster (*Ostrea edulis*)	22	7.70	Eymann et al. (2020)
Cortez oyster (*Crassostrea corteziensis*)	29	5.34	Guzmán-Agüero et al. (2013)
Blacklip Rock Oyster (*Saccostrea* lineage J)	24	15.20	Present study

Table 5. Range of 4 water quality parameters reported by a north Queensland prawn farm in 2019. Abbreviations as in Table 2

Water quality parameter	Mean	Minimum	Maximum
TSS (g l^{-1})	0.032	0.003	0.084
Chl *a* (µg l^{-1})	3.05	0.1	8.5
TN (µg l^{-1})	1136	290	2000
TP (µg l^{-1})	139	20	270

The net effect of oyster filtration on the effluent nutrient load reveals the exchange between nutrient uptake and faecal excretion (Jones & Preston 1999). The reduction of total phosphorus by 27% within the high-density treatment was likely due to the removal of phosphorus bound to organic and inorganic particulates. In comparison, the reduction of total nitrogen by 21% was less effective, which could be attributed to nitrogenous waste in oyster faeces, lessening the overall net reduction within the system.

The absence of significant settlement of suspended solids following the first hour, as demonstrated by the control treatment, suggests that much of the inorganic matter is primarily small particles which take longer to fall out of suspension than larger, heavier particles. This is in agreement with the findings of Ziemann et al. (1992), which highlighted that a high proportion of fine inorganic particles is characteristic of unlined earthen prawn ponds. During the process of filtration, oysters categorize particles based on size, weight and nutritional value. Organic particles and microalgae are typically ingested and retained as a source of food, while inorganic particles are ingested, flocculated together and then expelled as pseudofaeces through the inhalant opening (Tenore & Dunstan 1973, Dumbauld et al. 2009). Therefore, oysters can assist in the removal and settlement of small inorganic particles, as after flocculation particles are heavier and fall out of suspension faster (Jones & Preston 1999). This mechanism of reducing fine suspended solids appears to have been present in this study, as evidenced by the success of the high-density treatment in reducing TSS. Future studies could consider particle size analyses to quantify the distribution of particle sizes targeted by oysters.

In a previous study investigating the bioremediation ability of Sydney rock oysters *S. glomerata*, Jones & Preston (1999) highlighted that TN, TP, TSS and chl *a* from prawn effluent were all successfully reduced over a 2 h period in a static system with a stocking density of 0.7 oysters l^{-1} (Table 2). The percentage reduction of TN and TP appears to be comparable between that trial and the present study; however, the Sydney rock oyster trial returned a far greater reduction in chl *a*, while the current *Saccostrea* lineage J study returned a superior reduction in TSS (Table 2). It is important to note, however, that the effluent used in the current study contained over 3 times the level of chl *a*, while the effluent used in the Sydney rock oyster study contained over 5 times the level of TSS. High sediment loads have been shown to reduce or even inhibit oyster filtration and in some cases can lead to mortalities due to smothering (Hopkins et al. 1995). Further studies are required to assess the consequences of high sediment loads on oyster filtration rates and survival, in addition to settlement techniques that may reduce suspended silt prior to oyster filtration (Jones & Preston 1999).

Whilst a proportion of the suspended material filtered by oysters is excreted as either faeces or pseudofaeces, the retained particles are incorporated into oyster tissue. In order to eliminate these nutrients from the system completely, removal of the sediment and bacterial denitrification is required, as well as harvesting the oysters. In addition to the positive effects of oyster culture on factors such as turbidity, the net removal of nitrogen through harvesting can further assist in quantifying the ecosystem services provided by oyster farms within waterways (Dewey et al. 2011, Petersen et al. 2016). The mean harvest oyster weight of 75.4 g equates to a mass of 0.09 g of nitrogen stored within each oyster. When scaled up to a yearly *Saccostrea* lineage J harvest of 1 t (13 260 oysters), a total of 1.20 kg of nitrogen would be removed from the growout waterway. These findings are comparable to a similar study investigating the ability of *C. virginica* to take up and remove nitrogen from coastal estuaries. In that study, the mean percentage of nitrogen within oyster dry tissue was 8.6%, in comparison to 8.27% in *Saccostrea* lineage J specimens (Carmichael et al. 2012). Nitrogen content in bivalve tissues has been shown to depend on both species and location-specific attributes, including nitrogen load (Carmichael et al. 2012).

Recently, Tabrett et al. (2023) reported release monitoring data for 10 aquaculture farms in the Great Barrier Reef catchment between 2013 and 2022 and estimated approximately 113 t of total nitrogen released per yr, with an average TN of 2.25 mg l^{-1} and 1.51 mg l^{-1} released from barramundi and prawn farms, respectively. In 2020/21, the oyster industry in New South Wales harvested 5081 t of oysters valued at $58 270 492 AUD (NSW Department of Primary Industries 2022). If tropical oyster production in Queensland grew to this

scale it would remove approximately 6098 kg of nitrogen yr^{-1} from the system and contribute to a 6% reduction in total nitrogen yr^{-1} released from aquaculture farms in the Great Barrier Reef catchment, contributing to the Qld Government's Reef 2050 Plan (Tabrett et al. 2023). Farm based research trials are the next step required in this research to determine if *Saccostrea* lineage J oysters can be successfully grown from spat (5 mm) to adult oysters (70 mm) in a bioremediation setting and to confirm total nitrogen stores in these oysters over their lifetime.

Nutrient credit trading has been proposed as a strategy to achieve water quality improvement targets and provide supplementary income to those involved. Programs use a market-based approach and provide economic incentives to complete nutrient load and pollution reduction targets (Bricker et al. 2018). The process of nutrient trading enables point-source dischargers who reduce their nutrient discharge below target levels to then on-sell their surplus reductions or 'credits' to other dischargers who are unable to do so or face more expensive treatment options (Wijsman et al. 2019). A nutrient credit is a transferable unit that represents a volume of pollutant prevented from entering the environment and is defined by the difference between the discharge allowance and the measured discharge from that source (Wijsman et al. 2019). This process may lead to new income opportunities for farmers, businesses or investors who are able to quantify their net positive effect on waterway health (Petersen et al. 2016).

In Australia, an application of nutrient trading known as Reef Credits was developed in partnership with the Queensland Government's Reef 2050 Plan. Water quality targets in the plan for 2025 include a reduction in anthropogenic end of catchment loads of dissolved inorganic nitrogen by 60%, particulate nutrient loads by 20%, and fine sediment loads by 25%. Primarily involving stakeholders from the agricultural sector, Reef Credits aims to incentivise land users to refine practices and prioritise improvements to water quality. At present, the 2 primary goals of the Reef Credits scheme are targeting a reduction in sediment run-off through gully rehabilitation and a reduction in nutrient run-off through managed fertilizer application. The outcomes of this bioremediation study closely align with goals laid out by the Reef Credits scheme. This study has helped quantify the ability of oysters to take up and reduce TN, TP, TSS and chl *a*. This is a step towards evaluating the value of this species for the purpose of bioremediation. In future, this knowledge will hopefully provide an opportunity for primary production sectors in northern Australia to work collaboratively towards water quality improvements and diversify income sources.

There is considerable scope to further investigate the bioremediatory ability of the tropical blacklip rock oyster within a laboratory setting. Additional environmental parameters such as salinity, which can vary in intertidal tropical environments, should be investigated in future studies. Based on previous findings, the inclusion of a sedimentation phase prior to biofiltration will likely improve efficiency of oysters (Jones et al. 2001). When integrated within a flow-through system design, the maximum capacity of *Saccostrea* lineage J to reduce nutrients and suspended particulates is expected to be further enhanced.

An additional element that requires investigation is integrated multi-trophic aquaculture, with macroalgae grown in conjunction with oysters to assimilate dissolved nutrients and balance oyster excretion. Previous studies have highlighted that after sedimentation and oyster filtration have reduced suspended particulates, macroalgae can rapidly reduce dissolved nutrient loads from prawn culture effluent (Jones et al. 2001, 2002). As a form of integrated multi-trophic aquaculture, both oysters and macroalgae are highly marketable products with uses including human consumption and animal feed (Jones et al. 2001, Patel et al. 2021). As well as offering a potentially cheaper alternative to mechanical filtration and water treatment, polyculture provides an opportunity to diversify crops and create supplementary income. Subsequent *in situ* farm-based trials are the next step, to build on the findings from laboratory-based studies such as the present study and identify barriers to biofilter integration.

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Appendix.

Table A1. pH measurement taken during daily water quality monitoring throughout the acclimation period

	Treatment			
	20°C	24°C	28°C	32°C
Day 1	Collection	Collection	Collection	Collection
Day 2	8.11	8.11	8.13	8.15
Day 3	8.10	8.09	8.11	8.12
Day 4	8.08	8.08	8.12	8.11
Day 5	8.10	8.07	8.11	8.11
Day 6	8.08	8.06	8.09	8.08
Day 7	8.08	8.10	8.14	8.10

Table A2. Salinity measurements taken during daily water quality monitoring throughout the acclimation period

	Treatment			
	20°C	24°C	28°C	32°C
Day 1	Collection	Collection	Collection	Collection
Day 2	36	36	36	36
Day 3	36	36	36	36
Day 4	36	36	36	36
Day 5	36	36	36	36
Day 6	36	36	36	36
Day 7	36	36	36	36

Table A3. Mean dry tissue weight of *Saccostrea* lineage J, and the maximum and minimum temperature recorded for each treatment

Treatment (°C)	Mean dry tissue weight (g) ± SE	Maximum (°C)	Minimum (°C)	Variation (°C)
20	1.08 ± 0.117	20.8	20.6	0.2
24	1.02 ± 0.06	24.3	23.9	0.4
28	1.13 ± 0.07	27.8	27.3	0.5
32	1.06 ± 0.08	32.0	31.5	0.5

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