Seasonal changes in C, N and P budgets of green-lipped mussels *Perna viridis* and removal of nutrients from fish farming in Hong Kong

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ABSTRACT: Fish farming activities release substantial wastes, including uneaten fish feed, faecal pellets and dissolved excretory products, resulting in nutrient pollution and subsequent deterioration of water quality in surrounding waters. Owing to the nature of their high filtration rate and high population density, filter-feeding green-lipped mussels reared in fish culture waters can take up particulate matter with considerable efficiency; hence, the farming wastes are removed. The present study evaluated the feasibility and capacity of using the green-lipped mussel *Perna viridis* as a biofilter to remove farming wastes from fish rafts. Spats of green-lipped mussels were transplanted to artificial reefs deployed under fish farms in Hong Kong. After a 1 yr period of growth and acclimatization, the nutrient scope for growth (SFG) of the transplanted mussels was quantified *in situ* through a series of bimonthly measurements of carbon, nitrogen and phosphorus changes. The metabolic acquisition and expenditure of mussels exhibited considerable temporal fluctuation during the study period, owing to changes in exogenous environmental conditions such as food availability, water temperature and dissolved oxygen levels, and autogenous physiological status such as the reproductive cycle. For a standard *Perna viridis* of 70 mm shell length, the average SFG of carbon, nitrogen and phosphorus was 170.9, 18.6 and 4.3 µg h^{-1}, respectively. As a result, the annual nutrient assimilation capacities of a 70 mm mussel for carbon, nitrogen and phosphorus were 1476.9, 160.3 and 36.7 mg, respectively. Based on the practical culture density of mussels in southeast Asia, the assimilation capacity has the potential to remove fish farm wastes at mariculture sites.

KEY WORDS: Fish farming waste · *Perna viridis* · Filter-feeding · Biofiltration · Nutrient enrichment · Nutrient cycling

INTRODUCTION

The cultivation of marine fish underwent a rapid expansion in the last decades, with an annual growth rate of 10.5% from 1970 to 2002. As an illustration, the growth in per capita supply of food fish since 2000 was exclusively accounted for by the increase in aquaculture production (FAO 2004). Fish farming activities can, however, result in marine pollution owing to the release of organic and inorganic wastes from uneaten feed, faeces and dissolved excretory products (Pearson & Black 2001). The elevated nutrient levels derived from the farming activity stimulate the occurrence of nuisance and toxic algal blooms. The final decay of excess plant production consumes considerable dissolved oxygen (DO), which can lead to mass fish mortality even after the discontinuity of the discharge of farming waters (Wu 1995). As a result of changes in environmental conditions due to the impacts of farming wastes, subsequent alterations in infaunal (Gao et

al. 2005) and epifaunal (Cook et al. 2006) community structure occur in the fish culture and adjacent waters.

In aquaculture waters, the high nutrition content of the suspended fish-feed remains released from fish farms may be a potential food source for filter-feeding organisms. Use of such organisms as biofilters has proven to be one efficient measure by which to remove farming wastes from the fish culture zone without secondary pollution (Angel et al. 2002, Lefebvre et al. 2004). In an integrated mariculture system combining fish and filter-feeding animals, the 'biofilters' can utilize the organic wastes from the culture cages as food sources. The nutrient content in the organic matter is thus ingested and accumulated in the tissues of the organism instead of being dissolved in the water column or deposited on the seabed. Hence, the water quality can be improved (Haamer 1996, Gao et al. 2006).

Representatives of the Mytilidae (e.g. zebra mussels *Dreissena polymorpha* [Arnott & Vanni 1996] and blue mussels *Mytilus edulis* [Arnott & Vanii 1996]) are highly successful colonizers of inter- and sub-tidal habitats, playing a significant role in nutrient cycling and energy flow because of their dense population and filter-feeding mode (Smaal & Vonck 1997). Green-lipped mussels *Perna viridis* are widely distributed in tropical and subtropical areas of the Indo-Pacific region. In Hong Kong, *P. viridis* is a dominant euryhaline species from eastern oceanic to western estuarine waters (Huang et al. 1985). Thus, they are good candidates for biofilters that will remove organic wastes from fish culture cages. However, prior to their application, detailed dynamics of nutrient acquirements and expenditure by green lipped mussels in the fish culture zone should be elucidated in order to assess the efficacy of nutrient removal.

Polyculture combining salmonid farming and long-line rearing of mussels has been proposed and applied in practice (Lehtinen et al. 1998). This polyculture method provides an economic and environmental win-win resolution scheme that both reduces organic pollution and enhances shellfish production. However, the setting of long mussel ropes inside fish cages may inconvenience the fish farming operations in practice. In the present study, transplanted mussel ropes attached to artificial reefs deployed under fish rafts were proposed as biofiltration units in the fish culture zone. The objectives of this experiment were to (1) examine the metabolic seasonality of the green-lipped mussels cultured in the fish culture zone in response to fluctuations in environmental conditions, with the emphasis on the nutrient budget; and (2) evaluate the feasibility of applying the filter-feeding green-lipped mussels as biofilters to reduce farming waste at mariculture sites.

MATERIALS AND METHODS

Study area and sample treatment. The study area was a marine fish culture zone located in Kau Sai Bay, which is a semi-closed embayment in the eastern waters of Hong Kong (22° 21' N, 114° 19' E). There are no riverine inputs into the bay. The fish raft area—which was confined within the inner part of the bay—was approximately 4.6 ha, with water depth ranging from 12 to 16 m. Each fish cage was approximately $4 \times 4 \times 4$ m. The total fish stock was ~500 t, with an average density of 4.5 kg m^{-3}. Cultured species are mainly grouper *Epinephelus awoara*, snapper *Lutjanus russellii* and seabream *Acanthopagrus latus*. Small trash fish (mainly anchovies *Thryssa* spp.) are used as fish feed. Daily feed supply was 3 to 5 % of the total stock, i.e. 15 to 25 t d^{-1}.

In April 2002, rectangular artificial reefs (3 m length × 3 m width × 4 m height) made of cement concrete were deployed around the fish culture-zone boundary. Spats of green-lipped mussels with shell lengths of 25 to 35 mm were attached on ropes and hung alongside the artificial reefs. At each artificial reef, 12 lengths of 3 m rope with a mussel density of ~100 ind. m^{-1} were installed. An advantage of placing the mussel ropes under the fish raft instead of installing them inside the fish pans is that the ropes will not interfere with the fish farming activities. After a >1 yr period of growth and acclimation, 20 to 30 mussels with shell length of 45 to 90 mm were collected from the ropes by SCUBA divers at bimonthly intervals between August 2003 and June 2004. After collection, epibionts on the mussel shells were removed with caution to minimize disturbance to the experimental animals. After this treatment and before the determination of biofiltration capacity, the mussels were cultured *in situ* at a depth of 10 to 13 m—the same depth at which the artificial reefs were deployed—by being hung on a fish raft for 2 d and allowed to recover from possible changes in physiological state caused by sample collection and treatment.

Determination of mussel filtration. The filtration of mussels was determined via an indirect biodeposition method as described by Gao et al. (2002a) in a flowing system at the fish culture site. Seawater was pumped from the water depth where the artificial reefs were deployed. Each individual experimental mussel was kept in a separate beaker and supplied with continuously flowing seawater driven by a 10-channel pump. A beaker without an animal was used as a control. A preliminary experiment showed that a flow rate of 100 ml min^{-1} was appropriate in order to avoid recirculation of seawater filtered by mussels (Wong & Cheung 2003). Mussels were cultured for 2 to 3 h, depending on the amount of pseudofaeces and faeces produced.

During the experiment, 1 l seawater samples were collected from the control beaker at fixed time intervals of 30 min. The collected water samples were filtered through pre-combusted and weighed 47 mm Whatman GF/C filters and rinsed with isotonic ammonium acetate solution. The filters were then dried in an oven at 80°C for 24 h, weighed to the nearest 0.1 mg, ashed in a muffle furnace at 450°C for 6 h to combust the organic matter and reweighed to determine the total particulate matter (TPM; mg l^{-1}), particulate organic matter (POM; mg l^{-1}), particulate inorganic matter (PIM; mg l^{-1}) and organic content (f = POM/TPM ratio).

Pseudofaeces and faeces were cautiously collected during and at the end of the experiment whereby re-suspension of the faecal pellets was avoided. The total, organic and inorganic weights of faeces and psedofaeces were determined by the same method described for seawater samples. By dividing the production of pseudofaeces or faeces by the experimental time, the following rates were computed: total pseudofaeces rejection (RR; mg h^{-1}), organic matter rejection (ORR; mg h^{-1}), inorganic matter rejection (IRR; mg h^{-1}), total faeces egestion (ER; mg h^{-1}), organic matter egestion (OER; mg h^{-1}) and inorganic matter egestion (IER; mg h^{-1}).

Food processing rates (feeding and absorption) of the experimental mussels were calculated following Iglesias et al. (1992). Assuming that absorption of inorganic matter through the digestive system is negligible (Cranford & Grant 1990), the sum of IRR and IER might be considered to represent the rate of inorganic matter filtration (IFR; mg h^{-1}). Clearance rate (CR; l h^{-1}), which is defined as the volume of water cleared per unit time, was then estimated as CR = IFR/PIM. Filtration rate of TPM (FR; mg h^{-1}), which is defined as the food biomass filtered by the mussels per unit time, was computed as FR = CR × TPM, and filtration rate of POM (OFR; mg h^{-1}) as OFR = CR × POM. Ingestion rates of TPM (IR; mg h^{-1}) and POM (OIR; mg h^{-1}) were estimated as IR = FR − RR and OIR = OFR − ORR; hence, the f of absorbed food could be estimated as AR (mg h^{-1}) = OIR − OER, and absorption efficiency (AE) as AE = AR/OIR.

Determinations of oxygen consumption and nutrient excretion. To determine oxygen consumption (V$_{O2}$; μg h^{-1}), each mussel was placed in a sealed 500 ml glass chamber (experimental chamber). The sealed chambers and 2 other empty chambers without mussels (control chambers) were bathed in a large plastic tank supplied with seawater pumped continuously from the sea, in order to reduce the effects of temperature changes on the respiratory activities of the mussels. After 30 to 60 min, depending on the animal size, the DO levels of the experimental and control cham-

bers were measured with a DO meter (Yellow Springs Instrument). V$_{O2}$ was calculated using the following equation:

$$V_{O2} = (DO_C - DO_E) \times V/t$$

where DO$_C$ and DO$_E$ are the DO levels of the control and experimental chambers respectively, V the volume of chamber and t the experimental time.

For the tests of ammonium (V$_N$; μg h^{-1}) and phosphate (V$_P$; μg h^{-1}) excretion rates, mussels were maintained in separate glass beakers filled with 500 ml seawater for 30 to 60 min. Two additional empty beakers without mussels were used as control beakers. Water samples collected from the experimental and control beakers were stored in an icebox and taken back to the laboratory for nitrogen and phosphorus determination using the phenolhypochlorite and phospho-molybdate colorimetric methods, respectively, with a Flow Injection Analyzer (FIA; QuikChem 8000, Lachat Instruments) (Strickland & Parsons 1977). V$_N$ and V$_P$ were calculated using the following equation:

$$V_N \text{ (or } V_P) = (C_E - C_C) \times V/t$$

where C$_E$ and C$_C$ are the nutrient concentrations of the experimental and control beakers respectively, V the volume of beakers and t the experimental time.

Nutrient analysis for pseudofaeces, faeces and particulate matter. During each sampling occassion of the bimonthly field trips, seawater samples (1 l) were filtered through pre-combusted and weighed glass-fiber filters (Whatman GF/C) and dried at 80°C for 24 h to the nearest 0.1 mg and kept frozen at −80°C for future carbon, nitrogen and phosphorus analyses. Particulate organic carbon (POC; μg mg^{-1}) and particulate organic nitrogen (PON; μg mg^{-1}) of seston and faecal pellets on filter paper were measured with a CHNS/O Analyzer (PE2400 Series II, PerkinElmer) (Ribes et al. 2003). For POC measurement, carbonates were removed by fuming the moist residues retained on the filter paper over concentrated HCl (37 %) for 3 d. Samples were then re-dried and re-weighed. The percentage of carbon was corrected for the weight change owing to carbonate removal (Kristensen & Andersen 1987). Particulate organic phosphorus (POP) was determined following the wet digestion method. The particulate matter on the filter paper was digested with concentrated sulphuric acid (98 %) using a digestion block at 200°C for 30 min for digestion of detritus and 370°C for 2 h for digestion of other phosphorus constituents. Copper sulphate (CuSO$_4$) was used as a catalyst, and potassium sulphate (K$_2$SO$_4$) was added to raise the boiling point of the digesting acid. The concentration of POP was determined with a FIA (QuikChem 8000, Lachat Instruments).

Assimilation of nutrient elements. The filtration, oxygen consumption and nutrient excretion rates were size-standardized to the mean mussel shell length of 70 mm (~1 g tissue dry weight) according to the following equation (Gao et al. 2002b):

$$Y_S = (X_S/X_O)^b \times Y_O$$

where Y and X were the physiological parameters (feeding, oxygen consumption and nutrient excretion) and mussel shell length (mm) respectively, subscripts S and O represent the standard and observed values respectively, and b is the power coefficient obtained from the respective bimonthly allometric equations relating physiological parameters with shell length, i.e. $Y = aX^b$, where a is the regression coefficient.

The assimilation rates of the nutrient elements (C, N and P) were measured as scope for growth (SFG; µg h^{-1}) for each element, which was defined as the difference between acquisition and expenditure (Smaal & Widdows 1994). SFG of C, N or P could be calculated as:

SFG (µg h^{-1}) = AR – respiration or excretion rate

Oxygen consumption was converted to C excretion based on a mean respiratory quotient of 0.85: 1 µg $O_2 \equiv$ 0.32 µg C (Smaal & Vonck 1997). For C, excretion is zero, while for N and P, respiration is zero.

Assuming that the average SFG values were kept constant as measured values during the bimonthly test interval, the assimilation of the nutrients by the mussels was estimated as:

Assimilation (mg) = [SFG (µg h^{-1}) × 24 h × 60 d]10^{-3}

Data analysis. In order to determine how environmental factors such as food conditions, temperature, DO level and salinity affected the physiological processes of the mussels, regression analyses were ap-plied using simple linear or non-linear procedures, depending on the most appropriate function to be fit-ted in each case (Zar 1999). Residuals were analyzed to check the normality and constant variance of predicted dependents. All statistical procedures were performed with SPSS for Windows Release 14.0.

RESULTS

Hydrography, food conditions and nutrient contents

Bimonthly changes in the bottom hydrographic parameters (such as temperature [T; °C], DO [mg l^{-1}] and salinity [S; PSU]), food supply (in terms of TPM [mg l^{-1}], POM [mg l^{-1}], PIM [mg l^{-1}] and f [%]) and nutrient contents (including POC, PON and POP) of the suspended particulate matter are listed in Table 1. As shown in Fig. 1, water stratification oc-curred in August and exerted a considerable effect on bottom conditions. As a result of such stratifica-tion, a thermocline and a halocline existed from 4 to 10 m in August. Bottom temperature underwent sea-sonal variations, with high values (~26°C) recorded in October, decreasing gradually to the lowest recorded temperatures (~15°C) in February (Table 1). Because of the stratification, bottom temperature in summer (August) was even lower than in October despite the highest surface value in August (~31°C). Bottom DO levels were reasonably constant throughout the experimental period, except for the extremely low level in August that was a result of the stratification in summer. Bottom salinity was fully oceanic (~30 to 33 PSU) throughout the year; however, surface salin-ity in August fell to 26 PSU owing to the high sum-mer rainfall. TPM and PIM concentrations in winter were higher than in summer owing to the wave-

Table 1. Bimonthly changes in bottom hydrography (T, temperature; DO, dissolved oxygen, S, salinity), food conditions (TPM, total particulate matter; POM, particulate organic matter; PIM, particulate inorganic matter; f, organic content) and nutrient content of suspended particulate matter (POC, particulate organic carbon; PON, particulate organic nitrogen; POP, particulate organic phosphorus). With the exceptions of T, DO and S, data are mean ± 1 SD (n = 3–5)

| Parameters	Aug 03	Oct 03	Dec 03	Feb 04	Apr 04	Jun 04
T (°C)	23.6	25.9	21.8	15.0	18.9	21.8
DO (mg l^{-1})	1.7	5.7	6.1	7.2	5.9	6.7
S (PSU)	33.6	31.7	34.0	33.8	33.9	33.0
TPM (mg l^{-1})	10.68 ± 0.75	12.62 ± 0.88	15.15 ± 1.10	15.11 ± 0.18	12.19 ± 0.06	10.16 ± 0.06
POM (mg l^{-1})	6.17 ± 0.72	4.97 ± 0.15	4.30 ± 0.48	3.18 ± 0.22	3.92 ± 0.15	4.41 ± 0.57
PIM (mg l^{-1})	4.51 ± 0.27	7.65 ± 0.77	10.85 ± 1.32	11.29 ± 0.21	8.27 ± 0.06	5.75 ± 0.49
f (%)	0.58 ± 0.02	0.39 ± 0.02	0.28 ± 0.05	0.25 ± 0.03	0.32 ± 0.03	0.43 ± 0.08
POC (µg mg^{-1})	135.56 ± 10.36	81.33 ± 7.32	70.33 ± 8.36	81.64 ± 6.71	88.58 ± 7.02	129.73 ± 11.30
PON (µg mg^{-1})	24.56 ± 3.25	11.25 ± 1.53	13.22 ± 3.25	15.60 ± 1.02	9.32 ± 2.01	22.04 ± 2.31
POP (µg mg^{-1})	1.96 ± 0.03	1.69 ± 0.05	1.56 ± 0.03	2.32 ± 0.05	2.01 ± 0.03	2.29 ± 0.01
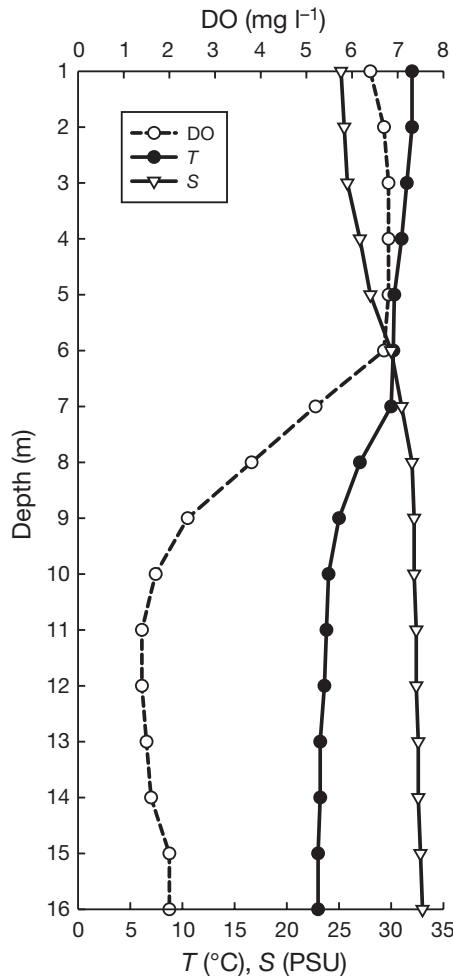
Fig. 1. Typical vertical profiles of temperature (T), salinity (S) and dissolved oxygen (DO) observed in the Kau Sai fish culture zone, Hong Kong in August 2003. Summer water stratification is apparent

Fig. 2. *Perna viridis.* Standardized clearance rate (SCR) of a 70 mm mussel vs. total particulate matter (TPM)

SCR was not significantly related to either POM or f, while SCR was negatively related to food quantity (in terms of TPM) following the equation:

$$SCR = 2.96 - 0.92 \times \ln TPM$$
$$(r^2 = 0.23, F_{1,153} = 46.67, p < 0.01)$$

(Fig. 2). Only 4 of 27 ind. rejected pseudofaeces throughout the experiment (all 4 in February 2004); hence, the results of pseudofaeces production are not presented. Standardized filtration rate (SFR) was independent of TPM and organic content (f). Standardized organic filtration rate (SOFR) was positively related to the food quality in terms of f according to the logarithmic model:

$$SOFR = 4.96 + 2.11 \ln f$$
$$(r^2 = 0.20, F_{1,153} = 34.00, p < 0.01)$$

Absorption efficiency (AE) was negatively related to the food quantity in terms of TPM:

$$AE = 0.92 - 0.037 \times TPM$$
$$(r^2 = 0.29, F_{1,153} = 62.1, p < 0.01)$$

(Fig. 3). Despite the decreasing AE with increasing TPM, AR was not related to either food quantity (TPM) or food quality (f).

driven re-suspension of sediment from seabed, leading to lower f in winter (December to February) than in June and August. POM, POC and PON exhibited higher values in the warmer season (June to August) and lower values in the colder months (Table 1). A seasonal pattern of fluctuation in POP levels was not obvious.

Filtration

In each of the 6 sampling months, the clearance rates of the experimental mussels were significantly related to individual size (shell length). The regressive allometric equations and the standardized CR (SCR) of a 70 mm medium-sized individual for each sampling month are listed in Table 2.
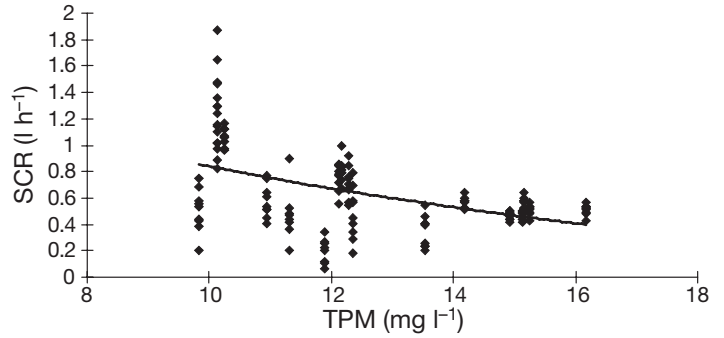
Table 2. *Perna viridis.* Allometric relationship of clearance rate (CR; l h^{-1}) to mussel shell length (SL; mm), and standardized CRs (SCR; l h^{-1}) of 70 mm mussels. For SCR, data are presented as mean ± 1 SD

Month	Equation	n	r^2	p	SCR
Aug 03	CR = 0.016 × SL$^{0.82}$	26	0.18	<0.05	0.52 ± 0.16
Oct 03	CR = 0.0002 × SL$^{1.69}$	26	0.18	<0.05	0.35 ± 0.18
Dec 03	CR = 0.24 × SL$^{0.64}$	26	0.46	<0.01	0.52 ± 0.06
Feb 04	CR = 0.55 × SL$^{0.43}$	27	0.26	<0.01	0.51 ± 0.06
Apr 04	CR = 0.43 × SL$^{0.45}$	26	0.55	<0.01	0.76 ± 0.11
Jun 04	CR = 0.31 × SL$^{0.70}$	24	0.57	<0.01	1.18 ± 0.25

Oxygen consumption and nutrient excretion

Oxygen consumption rate (V_{O2}) was significantly related to mussels' shell length following the allometric models. These models and the corresponding standardized oxygen consumption (SV_{O2}; μg h^{-1}), as well as the carbon respiration rate (V_C; μg h^{-1}) of a 70 mm mussel for each bimonthly interval, are listed in Table 3. The allometric relationships of nitrogen (V_N; μg h^{-1}) and phosphorus excretion rates (V_P; μg h^{-1}) to mussels' shell length, in addition to the corresponding standardized V_N (SV_N; μg h^{-1}) and V_P (SV_P; μg h^{-1}), are presented in Tables 4 & 5 respectively.

Oxygen consumption rate and nitrogen and phosphorus excretion rates were temporally synchronic, with higher metabolic rates apparent in the warm season (April to August) and lower rates in the cold season (October to February); however, they did not exhibit any significant relationships with food conditions in terms of either food quantity (TPM) or quality (*f*). All regression analyses showed that bottom temperature was the most dominant factor affecting the oxygen consumption and nutrient (N and P) excretion rates. SV_{O2} and SV_P were significantly correlated with bottom temperature (*T*) according to the following equations (Fig. 4a,b):

$$SV_{O2} = -10150 + 1099T - 26.4T^2$$
$$(r^2 = 0.29, F_{2,151} = 30.2, p < 0.01)$$
$$SV_P = 0.0005 \times T^{2.57}$$
$$(r^2 = 0.26, F_{1,151} = 54.1, p < 0.01)$$

With regard to nitrogen excretion, if the August data were excluded then SV_N was significantly related to temperature (Fig. 4c):

$$SV_N = -117 + 12.9T - 0.29T^2$$
$$(r^2 = 0.18, F_{2,125} = 13.8, p < 0.01)$$

Nutrient assimilation

Net budgets of the nutrients, including carbon, nitrogen and phosphorus (which were represented by scope for growth; SFG) and the total assimilation of these 3 nutrients by a standard 70 mm mussel are summarized in Table 6. In August 2003 and April 2004 the

Table 3. *Perna viridis.* Allometric relationship of oxygen consumption rate (V_{O2}; μg h^{-1}) with shell length (SL; mm), and corresponding standardized oxygen consumption rate (SV_{O2}; μg h^{-1}) and carbon respiration rate (SV_C; μg h^{-1}) of a 70 mm mussel for each experimental month. For SV_{O2} and SV_C, data are presented as mean ± 1 SD

Month	Equation	n	r^2	p	SV_{O2} (μg h^{-1})	SV_C (μg h^{-1})
Aug 03	$V_{O2} = 0.12 \times SL^{2.23}$	26	0.85	<0.01	1317 ± 222	421 ± 71
Oct 03	$V_{O2} = 0.64 \times SL^{1.59}$	26	0.52	<0.01	528 ± 178	169 ± 57
Dec 03	$V_{O2} = 0.014 \times SL^{2.45}$	26	0.81	<0.01	467 ± 187	149 ± 60
Feb 04	$V_{O2} = 0.019 \times SL^{2.38}$	27	0.79	<0.01	434 ± 192	139 ± 61
Apr 04	$V_{O2} = 1.13 \times SL^{1.62}$	26	0.56	<0.01	1148 ± 310	367 ± 99
Jun 04	$V_{O2} = 0.71 \times SL^{1.87}$	24	0.80	<0.01	2080 ± 463	666 ± 148

Table 4. *Perna viridis.* Allometric relationship of nitrogen excretion rate (V_N; μg h^{-1}) with shell length (SL; mm), and corresponding standardized nitrogen excretion rate (SV_N; μg h^{-1}) of a 70 mm mussel for each experimental month. For SV_N, data are presented as mean ± 1 SD

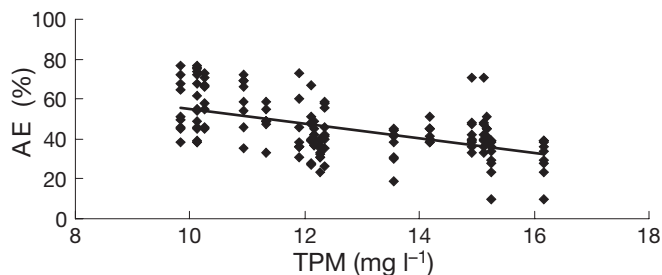| Month	Equation	n	r^2	p	SV_N (μg h^{-1})
Aug 03	$V_N = 0.0027 \times SL^{2.27}$	26	0.79	<0.01	39.8 ± 9.6
Oct 03	$V_N = 0.0019 \times SL^{2.22}$	26	0.77	<0.01	22.7 ± 13.9
Dec 03	$V_N = 0.0073 \times SL^{1.78}$	26	0.75	<0.01	12.5 ± 6.7
Feb 04	$V_N = 0.00025 \times SL^{2.59}$	27	0.57	<0.01	12.4 ± 6.1
Apr 04	$V_N = 0.015 \times SL^{1.85}$	26	0.63	<0.01	40.4 ± 8.3
Jun 04	$V_N = 0.016 \times SL^{1.81}$	24	0.84	<0.01	39.9 ± 13.2

Fig. 3. *Perna viridis.* Absorption efficiency (AE) vs. total particulate matter (TPM)

Table 5. *Perna viridis.* Allometric relationship of phosphorus excretion rate (V_P; μg h^{-1}) with shell length (SL; mm), and corresponding standardized phosphorus excretion rate (SV_P; μg h^{-1}) of a 70 mm mussel for each experimental month. For SV_P, data are presented as mean ± 1SD

Month	Equation	n	r^2	p	SV_P (μg h^{-1})
Aug 03	$V_P = 0.00094 \times SL^{2.02}$	26	0.59	<0.01	4.82 ± 0.76
Oct 03	$V_P = 0.00043 \times SL^{1.92}$	26	0.77	<0.01	1.29 ± 0.79
Dec 03	$V_P = 0.00031 \times SL^{1.88}$	26	0.81	<0.01	0.71 ± 0.70
Feb 04	$V_P = 0.000025 \times SL^{2.39}$	27	0.68	<0.01	0.71 ± 0.34
Apr 04	$V_P = 0.00066 \times SL^{1.94}$	26	0.82	<0.01	2.17 ± 0.33
Jun 04	$V_P = 0.00089 \times SL^{1.87}$	24	0.83	<0.01	2.11 ± 0.80

net budget of all 3 nutrients was negative, while in the other 4 months the budgets were positive, demonstrating that mussels could positively accumulate nutrients in 4 of the 6 sampling months. As a result, a medium-sized green-lipped mussel with a shell length of 70 mm can accumulate 1476.9 mg carbon, 160.3 mg nitrogen and 36.7 mg phosphorus each year.
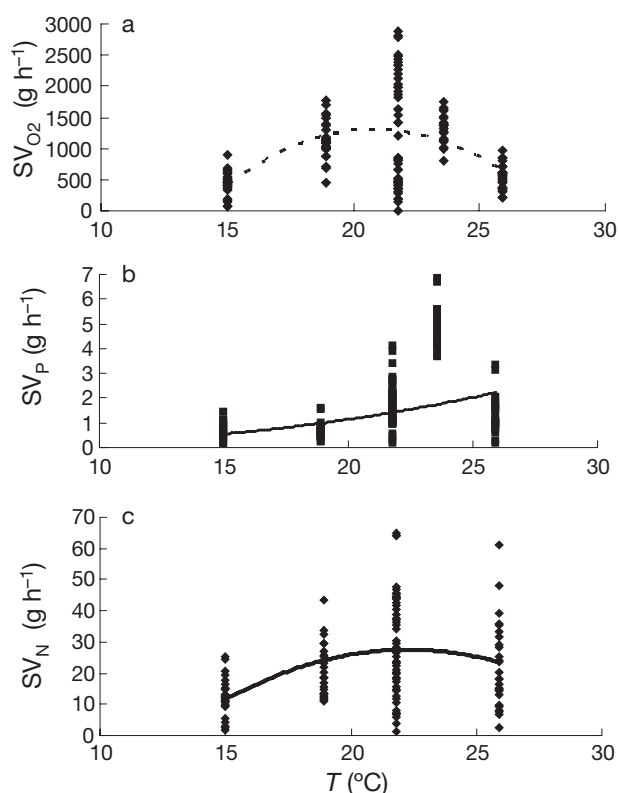

Fig. 4. *Perna viridis*. (a) Standardized oxygen consumption rate (SV_{O2}) vs. bottom temperature; (b) standardized phosphorus excretion rate (SV_P) vs. bottom temperature; (c) standardized nitrogen excretion rate (SV_N) vs. bottom temperature (data of August 2003 not included, see 'Results: Oxygen consumption and nutrient excretion'

Table 6. *Perna viridis*. Standardized net budget rate (SFG) and yearly assimilation of carbon, nitrogen and phosphorus by a 70 mm shell length mussel

Month	SFG ($\mu g\ h^{-1}\ ind.^{-1}$)			Assimilation (mg $ind.^{-1}$)		
	C	N	P	C	N	P
Aug 03	−60.2	−3.4	−0.4	−86.7	−4.9	−0.6
Oct 03	59.9	9.2	2.0	86.3	13.2	2.9
Dec 03	171.8	25.6	4.5	247.4	36.9	6.5
Feb 04	311.3	39.8	8.8	448.3	57.3	12.7
Apr 04	−27.6	−23.5	−0.2	−39.7	−33.8	−0.3
Jun 04	570.4	63.6	10.8	821.4	91.6	15.6
	Average SFG			Yearly assimilation		
	170.9	18.6	4.3	1476.9	160.3	36.7

DISCUSSION

Despite the low r^2 values of the equations relating various physiological rates to the environmental factors, the negative relationship of SCR to food quantity in terms of TPM demonstrated the pre-ingestive regulative ability of the mussels, which could adjust their feeding activity in response to variable conditions of food availability. Similar observations were reported for other filter-feeding organisms (e.g. Bayne et al. 1993, Bacon et al. 1998). With increasing TPM, mussels might actively reduce the filtered seawater volume. As a result of the adjustment of clearance rate, filtration rate could be kept independent of food conditions. Previous studies of mussels (Widdows et al. 1979), scallops and clams (Navarro et al. 1992, Bacon et al. 1998) have also shown that as particle concentration increases, food uptake is commonly regulated by a reduction in clearance rate. A similar reduction in clearance rates with increasing food availability was also recorded for local *Perna viridis* under both laboratory (Wong & Cheung 1999) and field conditions (Wong & Cheung 2001). The significant negative relationship of absorption efficiency (AE) to TPM indicated the post-ingestive regulative function during the feeding processes. Owing to the down-regulation of AE with increasing TPM, absorption rate was not related to either TPM or *f*, showing that in response to the fluctuation in food conditions, mussels might keep absorption relatively constant. Generally, absorption increases with increasing POM and achieves a maximum value at an intermediate value of POM, above which the absorption rate can be kept stable (Navarro et al. 1996, Wong & Cheung 1999).

The oxygen consumption rate and corresponding carbon respiration rate of *Perna viridis* demonstrated a seasonal pattern, with high values in the warm spring to hot summer season (April to August) and low values in the cool autumn to cold winter season (October to February). A similar pattern was recorded for a number of bivalves (Bayne & Newell 1983). Generally, temperature, food availability and reproductive condition are the major factors determining bivalves' oxygen consumption (Babarro et al. 2000). The significant regressive model between oxygen consumption and environmental temperature indicated that temperature is the most dominant factor affecting the respiration rate of this species. In general, oxygen consumption gradually increased with rising temperature from winter to late spring (December to June). The sudden drop in respiration in August and October relative to that in June was ascribed to physiological inhibition by hypoxia, which lasted from summer to early autumn despite the high water temperature in these 2 months (Norkko et al. 2005). The effect of food availability was

insignificant in the present study; however, many reports have demonstrated that oxygen consumption becomes reduced when food quality is low (Babarro et al. 2000) as a result of lower digestive activity and slower growth (Bayne et al. 1989). In this study, such a discrepancy may be due to small fluctuations in food availability. Reproductive cycling may be another factor controlling oxygen respiration. Significant correlations between oxygen consumption and reproductive activity have widely been reported (e.g. Iglesias & Navarro 1991, Smaal et al. 1997). The minimum oxygen demand in December and February coincided with the reproductive resting period during winter, and the abruptly increased metabolic rate (in terms of oxygen consumption) from April onwards might be driven by the nutritional requirement of gametogenesis or spawning after gonadal development in late spring (Lee 1988).

Both excretion rates of nitrogen from the metabolic product of protein and phosphorus from catabolism of phospholipid increased in spring (April) because glycogen reserves are largely used up by the mussels in winter, and more protein and lipid are exploited as internal energy sources in spring (Gabbott 1983). Nitrogen and phosphorus excretion exhibited a positive correlation with temperature as a result of increased physiological metabolism (Bayne & Newell 1983). Summer hypoxic conditions at the experimental site represented another important factor determining the metabolic behaviour of the mussels. Under conditions of low oxygen levels in the bottom layers owing to summer stratification, less energy was available to the mussels for food uptake. Consequently, metabolic requirements of mussels were met from internal sources with priority for glycogen reserves (Hawkins & Bayne 1985). With the reduction in glycogen reserves, more protein and lipid content were utilized. As a result, nitrogen and phosphorus excretion showed high values in August.

As a consequence of summer hypoxia in bottom waters at the experimental site, SFG of all nutrients including carbon, nitrogen and phosphorus exhibited negative values due to the hypoxic conditions in August. Negative nutrient SFG in April might be attributed to the consumption of nutritional reserves in winter and during spawning in spring (Smaal & Vonck 1997). In total, *Perna viridis* showed positive annual nutrient assimilation for carbon, nitrogen and phosphorus throughout the experimental period, indicating that green-lipped mussels cultured on the artificial reefs deployed at the fish culture site could efficiently accumulate nutrients. In the present study, we did not measure mussel growth concomitant with the physiological rates. However, previous studies validated the consistency of nutrient metabolism from SFG-based

calculation under laboratory conditions with that directly observed from tissue growth under field conditions (Smaal & Vonck 1997). Hence, this indirect measurement of nutrient accumulation in our experiment may well represent the real nutrient removal by green-lipped mussels in fish farms.

Previous studies have demonstrated the environmental advantages gained by cultivating filter-feeding species that help to counter increased levels of nutrients and particulate matter in fish culture zones and adjacent waters (Folke & Kautsky 1989, Porrello et al. 2003). Mussels are well known for their high filtering capability and for their occurrence in high densities of individuals (Gili & Coma 1998). They will not exhaust all nutrients in fish farms because other sources of nutrient input exist, including microbial N fixation, atmospheric nutrient deposition and mineralization of seabed sediment. The purpose of using mussels as biofilters in the present study was to reduce pollution resulting from farming wastes. A study by Leung et al. (1999) reported that annual nitrogen loss from a fish farm to surrounding waters was 321 g N kg^{-1} fish production. On average, annual production of sea-fish culture in Hong Kong per unit area is ~0.75 kg m^{-2} (data from Agriculture, Fisheries and Conservation Department, Hong Kong SAR Government), yielding $321 \times 0.75 = 241$ g N loss. The present study estimated that each mussel of medium size might assimilate 160 mg N. Hence, the theoretical density of mussel culture in fish farms required to counteract excess N input from farming activities is 241 g/160 mg, equivalent to 1506 ind. m^{-2}, which demonstrates that *Perna viridis* may be considered for use as biological cleansing units to mitigate organic pollution resulting from fish farming.

In Hong Kong, the growth of *Perna viridis* is highly seasonal and major growth is obtained within the period August to December (Cheung 1991). The most rapid annual growth rate for mussels of ~50 mm is recorded in their 1st year, and growth slows to ~24 and ~9 mm in their 2nd and 3rd year respectively. High mortality is observed after the maximum shell length (~90 mm) is obtained in the 3rd year (Cheung 1993). In addition, the present study recorded negative nutrient SFGs in April and August. Hence, the optimum timing for the transplantation of 1 yr old spats (<30 mm) to the artificial reefs would be after the spring spawning period (April) or after the adverse conditions in summer (August). To maximize the filtration potential of mussels as biofilters, a growth period of 3 yr prior to harvest is recommended.

It is noted that the monoculture of filter-feeding bivalves at high density generally enhances the sedimentation of suspended particulate matter from the water column to the sediments, resulting in accumula-

tion of organic matter in the benthic environment (Kaspar et al. 1985). However, the polyculture of carnivorous fish (e.g. grouper) and herbivorous/omnivorous species (e.g. green-lipped mussels) in the present study differs from the monoculture of filter-feeding bivalves. This is because the filter feeders in the polyculture system might reduce the settlement of wastes derived from fish farming, and nutrient concentrations in the biodeposits may be reduced prior to their sedimentation. Further monitoring at the present experimental site revealed that the concentrations of total organic carbon (TOC), total kjeldahl nitrogen (TKN) and total phosphorus (TP) of the sediment beneath the fish cages were significantly reduced from 53.3 mg g^{-1}, 4.4 mg g^{-1} and 7.9 mg g^{-1} to 43.3 mg g^{-1}, 2.4 mg g^{-1} and 2.6 mg g^{-1}, respectively, 2 yr after the introduction of mussels and other filter-feeding organisms on the artificial reefs (Gao 2005).

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