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Review

Using a macroalgae *Ulva pertusa* biofilter in a recirculating system for production of juvenile sea cucumber *Apostichopus japonicus*

Hua Wang [a,b], Chang-Fa Liu [a,b,*], Chuan-Xin Qin [a], Shu-Qing Cao [b], Jun Ding [b]

[a] *College of Marine Environmental Engineering, Dalian Fisheries University, Dalian 116023, PR China*
[b] *Key Lab of Mariculture and Biotechnology, Ministry of Agriculture, Dalian 116023, PR China*

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Abstract

A simple indoor recirculating system for production of juvenile sea cucumber (*Apostichopus japonicus*) was operated on a commercial scale for 90 days during winter. The system consists of three 70 m^3 sea cucumber rearing tanks and one biofilter tank where macroalgae (*Ulva pertusa*) was used as a biofilter in order to reduce water requirements. Effluent from the sea cucumber tanks drained into the macroalgae biofilter tank and were then returned to the sea cucumber tanks by a discontinuous-flow recirculation system. Survival and growth rates in the sea cucumber culture tanks were similar to those in the control tank (with one water exchange per day). The survival rate averaged about 87%. The average body weight increased from 3.5 ± 0.3 g to 8.1 ± 0.8 g and total sea cucumber biomass production over the experimental period was 745 g m^{-2} after initial stocking densities of 375 g m^{-2}. The growth rate of *U. pertusa* was 3.3% day^{-1}. *U. pertusa* was efficient in removing toxic ammonia and in maintaining the water quality within acceptable levels for sea cucumber culture; there were only small daily variations of temperature, pH and DO. The *U. pertusa* tank removed 68% of the TAN (total ammonia-nitrogen) and 26% of the orthophosphate from the sea cucumber culture effluent; the macroalgae biofilter removed ammonia at an average rate of 0.459 g N m^{-2} day^{-1}. It would be efficient to use the *U. pertusa* biofilter in a recirculating system for production of *A. japonicus* juveniles in winter.

Keywords: Sea cucumber; Production; Macroalgae; Recirculating system; Biofilter

Contents

* Corresponding author at: College of Marine Environmental Engineering, Dalian Fisheries University, Dalian 116023, PR China. Tel.: +86 411 84762700; fax: +86 411 84762700.
E-mail address: liucf@dlfu.edu.cn (C.-F. Liu).

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1. Introduction

Because it supplies good food value and economic value, sea cucumber culture has become an important industry in recent years in Dalian, PR China. Along the coastline, many traditional old shrimp ponds have been changed into sea cucumber culture ponds and many new ponds established. The area devoted to sea cucumber culture ponds has expanded rapidly, reaching more than 7000 ha in Dalian in 2004.

Apostichopus japonicus is the dominant sea cucumber species used in pond culture in Dalian. Before transferring *A. japonicus* to pond culture, indoor nursery processes during the winter aim to increase the juvenile survival rates. During this period, the average body length of sea cucumber can be increased from 1 cm to 3 cm (Liu et al., 2003).

Traditionally, normal flow-through a sea cucumber culture system needs one or two water exchanges per day to maintain water quality (Wang and Qin, 2000; Chang et al., 2003). In Dalian, the lowest winter seawater temperature is below 1 °C, so heating the water up to 10 °C is necessary to induce better survival and growth rates of *A. japonicus* juveniles. It has been suggested that the optimum temperature for juvenile *A. japonicus* ranges from 10 °C to 15.5 °C (Yu and Song, 1999; Dong et al., 2005), but heating large volumes of cold seawater to this temperature greatly increases operating costs. Coculture of abalone with sea cucumber can reduce water requirements at the laboratory scale (Kang et al., 2003), but this method cannot easily be applied on a commercial scale for production of sea cucumber juveniles. Other studies have focused on using fundamental biological and ecological information to improve production techniques for juvenile sea cucumber (Ramofafia et al., 1997; Battaglene and Seymour, 1998; Battaglene et al., 1999; Mercier et al., 1999, 2000; Uthicke and Karez, 1999; Yang et al., 2000; Michio et al., 2003).

Ammonia commonly needs to be removed from aquaculture systems, for it is toxic even at low concentrations; its safe levels in aquaculture ponds are lower than 0.02–0.3 mg L^{-1}. Accordingly, high water exchange rates have been applied in different aquaculture systems. Recirculating aquaculture systems, though they cost more, have attracted much attention because they consume less water than flow-through systems and also reduce water exchange rates (Shnel et al., 2002). Other studies have showed that nutrients have been efficiently removed from aquaculture wastewater using microalgae or macroalgae biofilters (Cohen and Neori, 1991; Neori et al., 1998; Chen, 2001; Abe et al., 2002; Schuenhoff et al., 2003). Various species of the genus *Ulva* have shown a high nutrient-uptake capacity, so they have been used for nutrient removal in many mariculture systems (Vandermeulen and Gordin, 1990; Cohen and Neori, 1991; Neori et al., 1998; Schuenhoff et al., 2003).

Water reuse methods require maintaining good water quality with a system that is easily operated at a low cost. Thus, we developed a simpler recirculating system for the production of juvenile sea cucumber *A. japonicus* on a commercial scale in winter. Local marine macroalgae *Ulva pertusa* were used as a biofilter to absorb the nutrients from the sea cucumber culture effluent. This system can reduce water requirements dramatically. In this study, the survival rate, growth rate, feed conversion and production of juvenile sea cucumbers were determined, the water quality of the recirculating system was examined, and the efficiency of the nutrient removal rate was calculated. The feasibility of using a discontinuous-flow recirculating system in *A. japonicus* juveniles culture is discussed.

2. Materials and methods

2.1. System design

The experiment was conducted in concrete tanks located inside a temperature-controlled building at Dalian Fisheries University, Dalian, China, during the overwintering period from 1 December 2004 to 28 February 2005. The sea cucumber-macroalgae biofilter concept was studied using a commercial scale recirculating system that included three 70 m^3 (7.00 m × 5.70 m × 1.75 m, $L \times W \times D$) sea cucumber rearing tanks (S1, S2, S3) and one (70 m^3) macroalgae biofilter tank (B1), each containing 60 m^3 of water. A discontinuous-flow recirculation system pumped water from the macroalgae biofilter tank into the S1, S2 and S3 sea cucumber culture tanks for only 8 h per day, from 8:00 to 16:00. The water flow into each sea cucumber tank was maintained at 200 L min^{-1}, and effluent from the sea cucumber tanks drained into the macroalgae biofilter tank by gravity. All organic material accumulated at the bottom and on the walls was removed and cleaned once a month. A mechanical blower aerated the water in each tank. Another 70 m^3 sea cucumber rearing tank (C1) was used as a control tank, with one water exchange per day.

2.2. Organisms

2.2.1. Sea cucumber stocking density and growth

Juvenile sea cucumbers (*A. japonicus*), 3.5 ± 0.3 g in body weight with a total biomass of 15 kg per tank, were purchased from a sea cucumber farm in Dalian, China. The juvenile sea cucumbers were hand-fed during the evenings with a commercial feed (25% protein, 8% lipid) at a rate of 3.5% of total body weight per day. Each month, the total sea cucumber biomass was weighed and the growth rate (GR) calculated:

$$GR = \frac{W_t - W_0}{t}$$

where t is the interval time (months); W_t the total sea cucumber wet weight at a given time (g); and W_0 is the initial sea cucumber total wet weight (g). The food conversion ratio (FCR) and the survival rate (SR) were calculated:

$$FCR = \frac{\text{total weight of dry food given}}{\text{total wet-weight gain}}$$

$$SR = \frac{W_t / BW_t}{W_0 / BW_0} \times 100$$

where BW_t is the final sea cucumber wet weight gain at a given time (g); and BW_0 is the initial sea cucumber wet weight (g).

2.2.2. Macroalgae growth

Approximately 40 kg of macroalgae *(U. pertusa)* were collected on the Heishijiao coast of Dalian. Air diffusers at the tank bottom suspended the macroalgae in the water column. The *U. pertusa* used were changed in the middle of the experiment, and their wet weight was then measured, and also at the end.

2.3. Water quality and nutrients

Water temperature, pH, dissolved oxygen (DO), salinity and light intensity were monitored daily at 09:00. Water from all tanks, filtered through a glass fiber filter (GF/F), was sampled every four or five days at 8:00, before the circulation period, and at 16:00, after the circulation period. In addition, one set of 24 h observation was carried out. Total ammonia-nitrogen (TAN), nitrite-nitrogen (NO_2^--N), nitrate-nitrogen (NO_3^--N), and orthophosphate (PO_4^{3-}-P) were analyzed using the phenate method, sulfanilamide-NED method, cadmium reduction method and ascorbic acid method (APHA et al., 1995).

3. Results

3.1. Abiotic parameters

Table 1 shows the mean, maxima, and minima for the water temperature, dissolved oxygen (DO), salinity and pH. The water temperatures in the sea cucumber culture tanks varied slightly due to the indoor recirculating culture system. Because of daily fluctuations in light intensity and the seasonal changes in the daily period of dark, the light intensity over the macroalgae biofilter tank ranged from 4 μmol m^{-2} s^{-1} to 78 μmol m^{-2} s^{-1}.

Table 1
Mean, minima and maxima for water temperature, salinity, dissolved oxygen (DO) and pH over the 90-day production period, measured at 09:00

Tank	Water temperature (°C)		Salinity (%)		Dissolved oxygen (mg L^{-1})		pH	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Culture tanks	10.8	10.1–11.5	30.0	28.0–31.0	7.55	7.28–8.14	8.18	7.97–8.47
Biofilter tank	11.0	10.1–12.0	30.0	28.0–31.0	8.14	7.55–8.60	8.22	7.94–8.52
Control tank	11.2	9.0–13.5	29.5	27.0–32.5	7.67	5.91–8.03	8.05	7.52–8.61

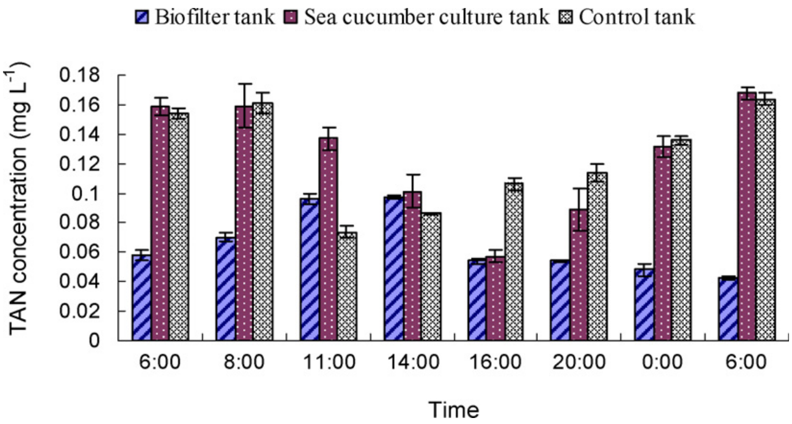

Fig. 1. Concentrations of ammonia in the tanks during the diurnal cycle.

3.2. Macroalgae growth and water quality

The *U. pertusa* yielded about 45 kg from 1 December to 15 January. The wet weight increased approximately 55 kg during the rest of the experimental period, with an average growth of 3.3% day^{-1}.

Concentrations of ammonia in the tanks during the diurnal cycle are shown in Fig. 1. The TAN concentration in the sea cucumber culture tanks fluctuated dramatically over the diurnal cycle, reaching its highest level at around 08:00 and its lowest concentration at 16:00. A slight increase of the TAN concentration in the macroalgae biofilter tank from 08:00 to 14:00 was generally followed by a decrease; under dark conditions, *U. pertusa* still absorbed ammonia, but with a lower uptake rate than during the day. Table 2 summarizes the mean, minimum and maximum values of TAN, nitrite, nitrate, and orthophosphate over the 90-day production period. The mean value of the TAN concentration in the sea cucumber culture tank at 08:00 was 0.151 mg N L^{-1} and the maximum just reached 0.189 mg N L^{-1}; then at 16:00 the mean value was 0.094 mg N L^{-1} and the peak value was 0.151 mg N L^{-1}; the overall mean concentration of TAN in the macroalgae biofilter tank is similar at 08:00 and 16:00. The mean values of nitrite and nitrate in the culture tanks were a little higher than in the control and macroalgae biofilter tanks. Orthophosphate values generally varied in all tanks throughout the experiment period. The results presented in this study suggested that inorganic nutrients did not accumulate in the sea cucumber culture tanks and the nutrient concentration remained low.

3.3. Growth performance of sea cucumber

Under the conditions of the indoor recirculating system, the juvenile sea cucumbers performed well throughout the 90-day culture period. No significant differences were found between the sea cucumber culture tanks and the control tank. The sea cucumber growth rate (GR), survival rate (SR) and food conversion ratio (FCR) are given in Table 3. The average body weight increased from 3.5 ± 0.3 g to

Table 2
Mean, minimum and maximum TAN, nitrite, nitrate and orthophosphate concentrations over the 90-day production periods

Tank	Mean and range							
	TAN (mg L^{-1})		Nitrite (mg L^{-1})		Nitrate (mg L^{-1})		O-phosphate (mg L^{-1})	
	08:00	16:00	08:00	16:00	08:00	16:00	08:00	16:00
Culture tanks	0.151 0.099–0.189	0.094 0.048–0.151	0.014 0.002–0.022	0.010 0.001–0.016	0.159 0.129–0.184	0.141 0.127–0.162	0.041 0.020–0.074	0.033 0.016–0.053
Biofilter tank	0.072 0.040–0.110	0.076 0.037–0.124	0.004 0.000–0.008	0.005 0.000–0.009	0.134 0.122–0.154	0.133 0.124–0.154	0.036 0.018–0.053	0.030 0.018–0.041
Control tank	0.161 0.145–0.210	0.126 0.086–0.164	0.005 0.001–0.011	0.005 0.001–0.012	0.135 0.120–0.178	0.136 0.121–0.180	0.037 0.016–0.064	0.032 0.016–0.058

Table 3
Growth parameters of the sea cucumber juveniles in the culture tanks and control tank

Tank	GR (g m^{-2} $month^{-1}$)			FCR			SR (%)
	1–31 December	1–31 January	1–28 February	1–31 December	1–31 January	1–28 February	1 December–28 February
Culture tanks							
S1	117	122	126	3.205	3.074	2.976	88
S2	114	119	123	3.289	3.165	3.057	86
S3	123	115	121	3.049	3.261	3.099	86
Control tank							
C1	116	113	117	3.233	3.319	3.196	87

8.1 ± 0.8 g, and total sea cucumber biomass production over the experimental period was 745 g m^{-2}, with stocking densities of 375 g m^{-2}. The growth rates (GR) during the experiment period were similar in each tank; they averaged 120 g m^{-2} $month^{-1}$ in the culture tanks and 115 g m^{-2} $month^{-1}$ in the control tank. The large variability in body wet weights between individuals creates difficulty in calculating the survival rate (SR). However, our large sample ($n = 250$) made a good estimate possible, the SR obtained in this study for each of the sea cucumber culture tanks was quite similar at about 87%, the same as for control tanks. The average food conversion ratio (FCR) in each culture tank was a little lower than in the control tank. The average total sea cucumber wet weight was above 29.79 kg in each culture tank and above 29.08 kg in the control tank.

3.4. Nutrient removal from the recirculating system

Fig. 2 shows the rate of ammonia removal in the seaweed culture tank during the experiment. The ammonia removal rate fluctuated between 2 December and 27 December; and the maximum removal rate, up to 0.758 g N m^{-2} day^{-1}, was observed on 31 December. After a slight decline from 5 January to 10 January, the ammonia removal rate began to drop significantly, while the ammonia concentration in the sea cucumber culture tank increased rapidly; subsequently, new *U. pertusa* were added to the biofilter tank. During the second experimental period, the ammonia removal rate in the culture tanks ranged from 0.368 g N m^{-2} day^{-1} to 0.635 g N m^{-2} day^{-1} (mean = 0.501 g N m^{-2} day^{-1}). The nutrient (N and P) removal rate and the removal efficiency in the recirculating system are summarized in Table 4. The *U. pertusa* tank removed 68% of the ammonia and 26% of the orthophosphate from the sea cucumber culture effluent.

4. Discussion

4.1. Water quality parameters

Maintaining an acceptable water quality is extremely important for the production of *A. japonicus* juveniles. A suitable water temperature for the growth of *A. japonicus* juveniles ranges from 0.5 °C to 30 °C and a

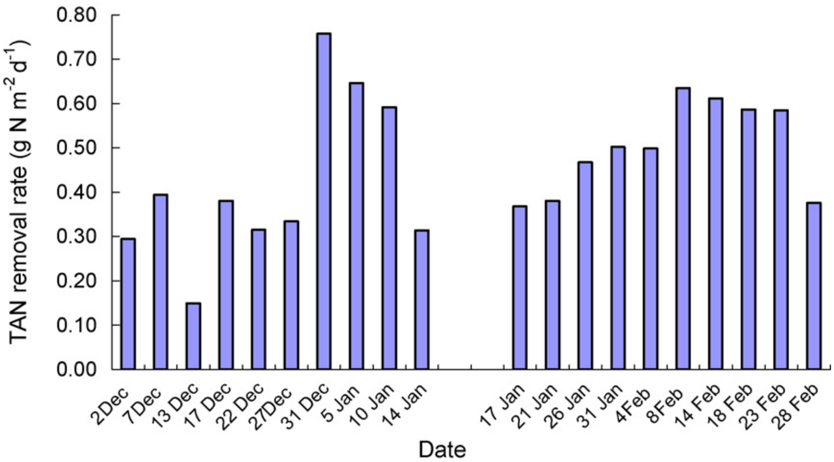

Fig. 2. Rate of ammonia removal in the seaweed culture tank during the experiment.

Table 4
Summary of nutrient (N and P) removal rate and removal efficiency in the recirculating system

Nutrient (N and P) removal	TAN	NO_2^--N	NO_3^--N	PO_4^{3-}-P
Removal rate (g N m^{-2} day^{-1} or g P m^{-2} day^{-1})				
Minimum	0.150	0.004	−0.040	0.008
Maximum	0.758	0.062	0.332	0.132
Mean	0.459	0.025	0.122	0.046
Removal efficiency (%)				
Minimum	23.43	5.49	−5.54	3.33
Maximum	109.31	122.86	40.48	65.05
Total removal efficiency (%)	67.60	40.11	17.07	26.12

recommended water temperature from 10 °C to 23 °C, with an optimum of 19–20 °C for 2 cm long and 10–15 °C for 5–15 cm long juveniles (Chang et al., 2004). The mean water temperature of 10.5 °C recorded in our study lies within these ideal limits.

It is generally agreed that the DO concentration is not critical to sea cucumber growth in an indoor tank culture system where aeration is used and oxygen saturation is stable during the experimental period. The salinity of the water in our recirculation system remained within a narrow range in all tanks throughout the experiment, and never negatively influenced the growth of the sea cucumber. The pH remained above 7.54 during the present investigation, but pH values were usually a little higher in the macroalgae tank than in the sea cucumber tanks, perhaps due to photosynthesis by the macroalgae.

In the present study, the concentrations of total ammonia-nitrogen (TAN), nitrite-nitrogen (NO_2^--N), nitrate-nitrogen (NO_3^--N) and orthophosphate (PO_4^{3-}-P) in the recirculating system remained at very low levels, and there were no significant differences among the tanks. The maximum values for TAN measured in the present experiment would be expected to have no effect on the growth and survival of the cultured sea cucumber.

4.2. Survival, growth, and production of sea cucumbers

The survival and growth performances of our *A. japonicus* juveniles were not below the values reported for *A. japonicus* juveniles and other species of sea cucumber in several other studies (Table 5). Dissimilarities in the culture species, the initial sizes of the individuals, and the experimental conditions preclude full comparison, but the survival and growth performance of our *A. japonicus* juveniles, similar in all tanks, were equal to or better those of previous researchers, such as Yu and Song (1999) and Chang et al. (2003).

Table 5
Summary of growth and survival of juvenile sea cucumbers

Species	Initial	Final	Growth rate	Survival rate	Comments	References
Actinopyga mauritiana	BW[a], 7.4 ± 0.2 g		10.4 ± 1.4 g $month^{-1}$		SD[b], 26 g m^{-2}	Ramofafia et al. (1997)
Holothuria scabra			0.2 ± 0.02 g day^{-1}	96	57 days, V = 140 L	Battaglene et al. (1999)
Stichopus japonicus	BW, 5.0 ± 1.2 g	BW, 18.35 ± 0.63g		100	n = 5, 90 days, V = 55 L	Kang et al. (2003).
Apostichopus japonicus	BL[c], 1.25 ± 0.5 cm			63.5	14 months, pond culture	Yu and Song (1999)
Apostichopus japonicus	BW, 16.7 ± 5.24 g	BW, 25.8 ± 6.45g		100	SD, 50 g m^{-2}, 48 days	Yang et al. (2000)
Apostichopus japonicus				60–90	Indoor culture	Chang et al. (2003)
Apostichopus japonicus	BW, 4.54 ± 0.38 g	BW, 11.81 ± 3.15 g	(2.72 ± 0.75)% day^{-1}	100	n = 5, 36 days, V = 40 L	Dong et al. (2005)
Apostichopus japonicus	BW, 3.5 ± 0.3 g	BW, 8.1 ± 0.8 g	118 ± 6 g $month^{-1}$	87	SD, 375 g m^{-2}, 90 days	Present study (2005)

[a] Sea cucumber wet body weight.
[b] Sea cucumber stocking density.
[c] Sea cucumber body length.

Our indoor recirculating system provided the sea cucumbers with adequate growth conditions. The growth rate of *A. japonicus* is dependent on relatively high quality and stable water conditions, especially the latter, with optimum growth ranging between 10 °C and 15 °C (Yu and Song, 1999; Dong et al., 2005).

An additional factor that may have accelerated the growth of our *A. japonicus* was the supply of artificial food without removal of food residues or feces. Ramofafia et al. (1997) and Kang et al. (2003) found that siphoning feces out of the tank reduced the growth of the animals, in a system where diatoms and bacteria covering the bottom of the tank possibly supplied additional feed for sea cucumbers. Our sea cucumbers might also have benefited from such enrichment of their food by bacteria or diatoms.

Our *A. japonicus* juveniles were hand-fed at a rate of 3.5% of total body weight per day, and showed a lower FCR than those fed 5–10% of total body weight per day (Chang et al., 2004); our low FCR values should reduce the costs of sea cucumber culture and improve water quality control.

Sea cucumbers survive well in concrete tanks with a continuous supply of seawater (Ramofafia et al., 1997; Yang et al., 2000; Dong et al., 2005); however, these systems need large amounts of fresh seawater. The amount of water consumed was small in our recirculating system. In our control tank, one cubic metre of seawater could only produce 2.56 g sea cucumber, whereas in our recirculating culture tank, one cubic metre of seawater could produce 80 g sea cucumber.

4.3. Macroalgae performance and nutrient removal

Accumulation of inorganic nitrogen, particularly ammonia-nitrogen, is the most common water quality problem in aquaculture. Compared to either a water flow-through method or an integrated water-purification treatment, using the macroalgae *Ulva* as a biofilter in an intensive aquaculture system is considered the most effective and feasible method. In intensive fishpond systems, an *Ulva* stocking density of 1.0 kg m^{-2} as suggested by several studies (Debusk et al., 1986; Neori et al., 1991) may have improved growth performance. *Ulva pertusa* yields have generally been lower (Neori et al., 1991, 1998; Schuenhoff et al., 2003); its relatively poor growth may be associated with lower water exchange rates, poor light penetration, and low nutrient concentrations in the recirculating system (Debusk et al., 1986; Vandermeulen and Gordin, 1990; Neori et al., 1998).

But the high nutrient removal rate in the present study suggests that *Ulva pertusa* might adapt to our experimental conditions. We compared nutrient-uptake rates among algal species in integrated aquaculture systems, as follows. *Ulva lactuca* showed a mean ammonia-N removal rate of 49–56% at fluxes of 4.8–5.2 g m^{-2} day^{-1} from marine fishpond effluents (Cohen and Neori, 1991); an abalone and macroalgae culture system (Neori et al., 1998) removed 55% of ammonia-N at flux of 4 g m^{-2} day^{-1}. -1. The ammonia nitrogen removal efficiency in the present study seems to be similar to that reported by Schuenhoff et al. (2003, 64% TAN removal) for an integrated fish and seaweed (*Ulva lactuca*) system. Our study also showed a high maximum nitrate removal efficiency of 40% and a total nitrate removal efficiency of 17%. The removal efficiency of orthophosphate in our macroalgae biofilter tank was similar to that in other studies (Cohen and Neori, 1991; Neori et al., 1998; Schuenhoff et al., 2003). Evidently, nutrient concentrations in a recirculating system can be maintained at low levels.

Overall, *A. japonicus* juveniles in a recirculating system showed similar survival and growth rates to those cultured in flow-through systems that maintain stable water temperatures and water quality. *U. pertusa* are cheap and easy to obtain. We have shown that cultivation of *U. pertusa* in a recirculating system is efficient in removing toxic ammonia and in maintaining the water quality within acceptable levels for sea cucumber culture; consequently, it can reduce water requirements and reduce the operating costs when it is necessary to heat large amounts of cold seawater. In conclusion, it is efficient to use a *U. pertusa* biofilter in a recirculating system for production of *A. japonicus* juveniles in winter.

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