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A semi-recirculating, integrated system for the culture of fish and seaweed

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Abstract

Biofiltration allows for environmentally sustainable mariculture. An intensive, biofiltered recirculating integrated system producing fish and seaweed on a semi-commercial scale was evaluated with respect to production and to nutrient and heat budgets. The system consisted of abalone (*Haliotis discus hannai*) and sea urchin (*Paracentrotus lividus*) tanks, an intensive fishpond (*Sparus aurata*), and a three-stage *Ulva lactuca* biofilter, which cleaned and recirculated 50% of the effluent back to the fishpond. To preserve water heat, the shellfish and fishpond units were both covered with greenhouses; the biofilter unit was covered with a greenhouse only during winter. Seaweed yield was variable and averaged 94 and 117 g m^{-2} day^{-1} (fresh weight) in periods with and without greenhouse cover, respectively. Protein content of *U. lactuca* averaged above 34% of dry weight. The biofiltration of only 50% of the water through the seaweed biofilter reduced the export of dissolved nutrients to the environment by nearly 30%. Peak ammonia excretion by the morning-fed fish coincided with maximum seaweed light-dependent ammonia uptake and concentrations of ammonia in the fishpond remained within nontoxic limits. Also, daytime photosynthesis of *U. lactuca* (uptake of CO_2) met fish respiration (production of CO_2), thus balancing fishpond pH levels within safe limits regarding ammonia toxicity. Daytime oxygen demand by the fish was partially met by the photosynthetically generated oxygen. Before covering the biofilter with a greenhouse, it lost much heat, reducing the temperature in the fishpond. Following the greenhouse covering of the biofilter, heat loss ceased and consequently the fishpond temperature was raised. Recirculation through the biofilter improved system sustainability; it reduced

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water use, lowered negative environmental impact, and maintained stable and safe water quality conditions in the fishpond.
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1. Introduction

Sea bream, like several other finfish, retain below 30% of feed nitrogen. In conventional cage mariculture and flow-through fishponds, the excess nutrients enter the environment as dissolved ammonia, faeces and uneaten feed (Porter et al., 1987; Krom and Neori, 1989; Lupatsch and Kissil, 1998). Nutrient-rich effluents cause rising concern due to eutrophication of coastal waters (Ziemann et al., 1992).

In land-based mariculture, water quality can be controlled by either a high rate of water exchange, which is costly and environmentally very similar to marine cages or by water treatment and subsequent recirculation, which also comes at a price (Zucker and Anderson, 1999). To offset treatment cost, it is necessary to increase the income from the system and reduce operational costs. This is achievable by integrated mariculture, which uses the excess nutrients to diversify the fish farm's products and provides a more efficient use of resources (Muir, 1996).

In an integrated system, the excretion of intensively raised organisms such as fish or shrimp, with the aid of solar energy, is converted into valuable algal biomass. Each species is cultured in a separate module, permitting optimisation of culture conditions for each organism. The seaweed produced may create a separate income by serving as feed for shellfish, by being used as a feed constituent, or even as an increasingly popular ingredient in East Asian cuisine. Previous research on a smaller scale has demonstrated the biological feasibility of fishpond water recirculation via seaweed biofilters (Neori et al., 1991, 1996) and the subsequent seaweed use for gastropod culture (Neori and Shpigel, 1999; Neori et al., 2000). Grown under a high nutrient flux, the cultured seaweed (*Ulva lactuca*) contained over 35% protein (DW) and was shown to be a suitable food for abalone (Shpigel et al., 1998, 2000). Recirculation of fishpond effluents through seaweed biofilters raises concerns about heat loss in winter, which may reduce fish growth (Lupatsch and Kissil, 1998) and ammonia toxicity, especially at photosynthetically induced high pH (Wajsbrot et al., 1991, 1993). On the positive side, apart from removing ammonia, the seaweed may contribute to the oxygen budget of the fishpond and reduce the level of CO_2. However, to be commercially implemented, novel concepts should be scientifically demonstrated on a commercially relevant scale.

In the present study, we evaluated the performance of a commercial-scale fishpond with 50% of its effluents recirculated through *U. lactuca* biofilters during autumn and winter. This was done with respect to overall and diurnal budgets of nutrients, levels of dissolved oxygen and water pH, and the effect of greenhouse use on water temperature.

2. Materials and methods

2.1. Experimental setup

The integrated system (Fig. 1) consisted of an intensive fishpond stocked with adult gilthead sea bream (*Sparus aurata*) and a biofilter with the seaweed *U. lactuca*. Fresh seawater first entered twelve 0.8 m^3 tanks containing shellfish (*Haliotis discus hannai* or *Paracentrotus lividus*) that were fed with *U. lactuca*. The effluent from this unit (4 m^3 h^{-1}), together with recycled water from the biofilter described below (4 m^3 h^{-1}) entered a greenhouse-covered (with Green PVC) fishpond, aerated by two aspirators. The fishpond effluent (8 m^3 h^{-1}) was screened with an automatic particle filter (400 μm; Compact Air 2''®, Israel) and the separated particles were channeled to a large settling pond stocked with Tilapia. Half of the screened effluent (4 m^3 h^{-1}) recirculated through the seaweed biofilter, back into the fishpond as described above.

In the biofilter units, unattached thalli of *U. lactuca* were kept suspended by aeration. The shallow ponds were arranged in a series (to be described elsewhere) with an overall surface area of 43.7 m^2 and a volume of 12 m^3. Water flowed from one pond to the next through 3-mm mesh screens and standpipes. The biofilter design (Fig. 1) was aimed at improving the protein level of the seaweed along with maintaining high nutrient uptake efficiency (Msuya et al., in preparation). After every harvest, the seaweed was restocked at a density of 1 kg m^{-2} to the same pond while the surplus was noted as the yield.

2.2. Experimental periods

Recirculation through the seaweed biofilter was running from November to March. A transparent greenhouse, covering the biofilter was constructed during the first days of

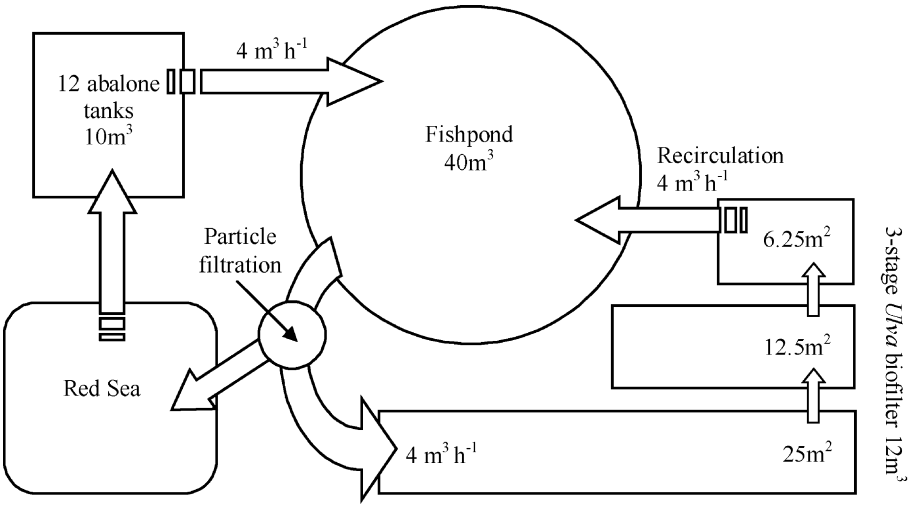


Fig. 1. Scheme of the semi-recirculating integrated system.

January. Intensive sampling programs were carried out in two separate periods. During other times, the system was monitored less frequently. The first intensive sampling period took place over 51 days (6 November–27 December, 2000), with first weekly, then fortnightly harvests of the seaweed. After the seaweed biofilter was covered by a greenhouse, a second intensive sampling period took place over 28 days (from 21 February to 21 March, 2001), with weekly seaweed harvests. Harvest days occurring when system operation was interrupted were not counted in the nutrient budget calculations described below.

2.3. Experimental procedures and calculations

Initially, 1226 adult sea bream (*S. aurata*) at a biomass of 11.5 kg m^{-3} were stocked in the fishpond. At the beginning and the end of each intensive sampling period, 200 randomly chosen individual fish were weighed.

The fish were manually fed to satiation with a commercial feed (Matmor, Evtach Israel: 45% protein, 19% lipid and 1.4% phosphorus) during the morning hours, to match the period of highest nutrient excretion (Porter et al., 1987) with the expected maximum uptake in the biofilter during photosynthetically active hours. *S. aurata* grown on a diet of this feed is reported to contain 28.5 g nitrogen (N) and 7.2 g phosphorus (P) on average per kilogram live weight (Lupatsch and Kissil, 1998). These values were used in the nutrient budgets to estimate the amounts of N and P retained in fish growth.

Growth rate and yield of *U. lactuca* were measured following the procedures outlined in Neori et al. (1991). The protein content of the seaweed was determined by Kjeldahl analysis, while the level of phosphorus was analysed by ashing samples and applying the vanado–molybdate method (AOAC, 1980). N and P incorporated into seaweed biomass were calculated according to these results.

Water flow rates between the system units were monitored with electromagnetic flow meters (Arad Dalia, Israel). Calibrated temperature sensors (Tecknoma®) were located in the effluent of the abalone tanks, fishpond, and both ends of the biofilter. Both types of devices were connected to a computer, permitting the automatic storage of hourly data.

Water samples for dissolved nutrients—total ammonia nitrogen (TAN), nitrite and nitrate (ToxN), and orthophosphate (*o*-phosphate)—were taken at four points: the influent to the fishpond from the abalone tanks, the fishpond's effluent, and both ends of the biofilter. Samples were collected in acid-rinsed 500 ml plastic jars and filtered (GF/C Whatman). They were refrigerated for up to a day until analysis. After an initial period of preliminary measurements to determine optimal sampling times, a weekly sampling scheme was carried out. Duplicate nutrient concentration analyses were made with a Technikon Autoanalyzer II. TAN analysis followed the method described in Krom et al. (1985) and Porter et al. (1987). Levels of ToxN were determined after Solarzano and Sharp (1980) and phosphate was analysed according to Krom et al. (1995). Water flow rates between the system components together with the nutrient concentrations were used in the calculation of steady-state nutrient budgets (Fig. 2).

On weekdays, at the points of nutrient sampling, dissolved oxygen (DO) and pH levels were determined with hand-held devices (OxyGuard® and Checker–Hanna Instruments, respectively) that were calibrated daily according to their respective manuals. The

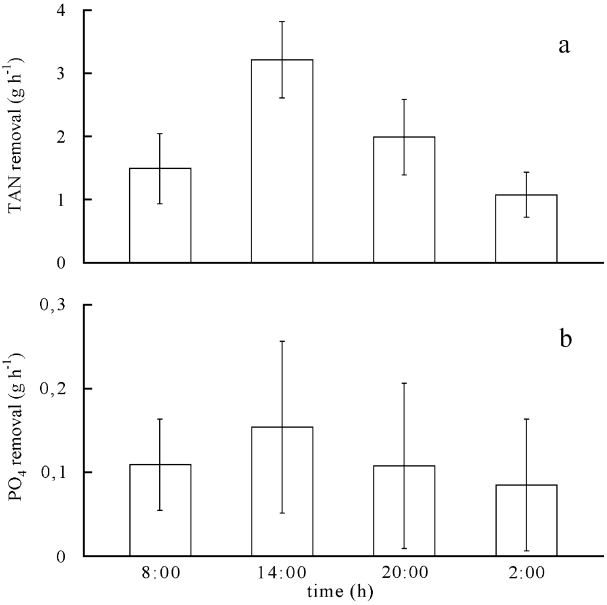


Fig. 2. Averages of hourly uptake rates of total ammonia nitrogen (a) and orthophosphate (b) at sampling times in the biofilter (error bars: ± SD).

meteorological station at Eilat Airport kindly provided hourly air temperature data. The 24 h extremes of air temperature inside and outside the greenhouse were recorded from a logging thermometer. Water temperatures and flow rates were used to calculate heat budgets as per the following equations.

Hourly energy input by water flow (kcal h^{-1}):

$$q_{in} = C_w \Phi_w Q(T_{in} - T_{out})\ 1000^{-1}\ 4.19^{-1}$$

where C_w = water heat capacity constant (J kg^{-1} K^{-1}); Φ_w = water density (kg m^{-3}); Q = flow rate (m^3 h^{-1}); T = water temperature (°C); 1000 = conversion factor for kcal; 4.19 = conversion factor from joule to calories.

Combined fishpond influent temperature:

$$T_{in\ fish} = ((T_r Q_r) + (T_a Q_a))(Q_r + Q_a)^{-1}$$

where r = recirculation; a = from abalone tanks.

Water heat (q) in kcal, assuming the effluent temperature is constant throughout the unit:

$$q = M_w T_{out}$$

where M_w = water mass of either biofilter or fishpond.

Change in water heat per hour:

$$\Delta q = q_{t_2} - q_{t_1}$$

where t_1 and t_2 stand for data 1 h apart.

Influence of unknown fluxes such as evaporation, long wave and solar radiation, and wall conduction on the hourly change in water temperature:

$$\Delta q_x = \Delta q - q_{\text{in}} \quad (1)$$

Subsequently, to obtain values of kcal h^{-1} m^{-2}, results for Eq. (1) of biofilter and fishpond were divided by the respective surface areas.

3. Results

3.1. Nutrient budget

The biofilter produced an *U. lactuca* yield with a high level of protein (Table 1). Seaweed yield was always substantial but differed between harvests. On average, yield (FW) was 94 and 117 g m^{-2} day^{-1} in the first and second sampling periods, respectively, and the molar N/P ratio of the seaweed yields averaged around 35 and 27, respectively. During both experimental periods, *U. lactuca* yield totaled 310 kg (FW). Since half of the fishpond effluent was discharged after particle filtration, only half of the dissolved nutrients were passed on to the biofilter. Chemical analyses of the seaweed reveal that the biofilter removed 1542 and 678 g N, in the first and second period, respectively. Likewise, 92 and 57 g *o*-phosphate, respectively, became incorporated into the seaweed (Table 1).

In both experimental periods (see Table 2), the fish consumed a total of 175 kg of feed, containing 12.6 kg N and 2.5 kg P. The fish grew by 52 and 27 kg, incorporating 20% and 15% of feed nitrogen, in the first and second periods, respectively. Likewise, 26% and 19%, respectively, of phosphorus from the feed was locked up in new fish biomass. Initially, these nutrient retention efficiencies were close to expected levels for fish of this size (Lupatsch and Kissil, 1998), but in the second period they decreased.

Apart from nutrients retained in growth, 17% of N input and 52% of P input were found in the faeces. The remaining nutrients were excreted and left the fishpond in dissolved forms, with 50% being passed on to the biofilter, as described above.

The actual concentrations of dissolved inorganic nitrogen were similar to those anticipated by the sea bream nutrient budgets described above, while the concentrations of *o*-phosphate were much higher than expected (Table 2). In the beginning, calculated amounts of TAN were very close to measured values but later on, the concentrations of ToxN rose and, as explained by Neori (1996), it was not removed by *U. lactuca* (Fig. 3).

Table 1
Properties of *U. lactuca* at each harvest during the two experimental periods

Date	Yield		Dry matter (%)	Protein (N × 6.25) (% of DM)	N (g) uptake	P (% of DM)	P (g) uptake	N/P ratio (molar)
	(kg FW)	(g FW m^{-2} day^{-1})						
First period, no greenhouse								
6 November, 2000			12	38		0.48		28
15 November, 2000	34.5	100	12	40	265	0.46	19.0	29
29 November, 2000	53	85	14	42	499	0.5	37.1	31
13 December, 2000	32	63	12	43	264	0.38	14.6	40.5
27 December, 2000	67	127	12	40	515	0.27	21.7	47
Total	187				1542		92.4	
Mean	46.6	93.8	12.4	40.6		0.4		35.1
± SD	16.5	26.9	0.9	1.9		0.1		8.3
U. lactuca incorporation rate (mg m^{-2} day^{-1})					751		45	
Second period, with greenhouse								
21 February, 2001			11	35		0.44		28
28 February, 2001	36	142	10	29	167	0.4	14.4	26
07 March, 2001	34	132	9	32	157	0.46	14.1	25
14 March, 2001	26	91	11	37	169	0.48	13.7	27
21 March, 2001	27	103	11	39	185	0.48	14.3	29
Total	123				678		56.5	
Mean	30.8	117.0	10.4	34.4		0.5		27.0
± SD	5.0	24.0	0.9	4.0		0.0		1.6
U. lactuca incorporation rate (mg m^{-2} day^{-1})					646		54	

Table 2
Nutrient (N and P) budgets for the two experimental periods (numbers rounded off)

Period		#1		#2	
Days of closely monitored feeding and recirculation		47		24	
Food input (kg)		103		72	
Fish biomass increase (kg)		52		27	
FCR (kg feed/kg weight gain)		1.98		2.66	
		g	(%)	g	(%)
Nutrients in food	N	7416	100	5184	100
	P	1442	100	1008	100
Nutrients in new fish biomass	N	1482	20	770	15
	P	374	26	196	19
Nutrients in particulate matter[a]	N	1261	17	881	17
	P	750	52	524	52
Dissolved nutrients					
In the fishpond effluent					
Estimated[a]	N	4673	63	3533	68
Measured: TAN + ToxN	N	4760	64	3670	71
Estimated[a]	P	318	22	290	29
Measured: *o*-phosphate	P	760	52	640	63
Passed on to biofilter[b]					
Estimated[a]	N	2349	32	1766	34
Measured	N	2305	31	950	18
Estimated[a]	P	161	11	144	14
Measured	P	380	26	320	32
Incorporation into biomass of *U. lactuca*	N	1542		678	
Fraction of estimated[a] available TAN			66		38
Fraction of measured available TAN			67		71
	P	92		57	
Fraction of estimated[a] available *o*-phosphate			57		40
Fraction of measured available *o*-phosphate			24		18
Loss of dissolved nutrients from the system					
TAN		2854	38	1153	22
ToxN		300	4	1700	33
o-phosphate		380	26	320	32

[a] According to Lupatsch and Kissil (1998), *S. aurata*, fed to satiation with the locally available feed, excretes 17% of feed nitrogen as particulate matter in the faeces. Subtracting this value and retention in growth from total feed nitrogen yields, the level of total dissolved nitrogen. Similarly, 52% of phosphorus is excreted as particulate matter. Note the large discrepancy between estimated and measured values for dissolved P and for rates of nutrient uptake.

[b] Only 50% of fishpond effluent recirculated through the biofilter; levels of ToxN within this water were not utilised by *U. lactuca* and are therefore excluded.

Less TAN (available to seaweed uptake) was passed on to the biofilter in the second period than in the first (Table 2). According to the seaweed composition data, the biofilter removed around 70% of available TAN and only about 20% of available *o*-phosphate (Table 2). With the nutrient concentration data from an intensive 24 h sampling regime (Table 3), the biofilter removed 66% of available TAN, 20% of available *o*-phosphate and no ToxN.

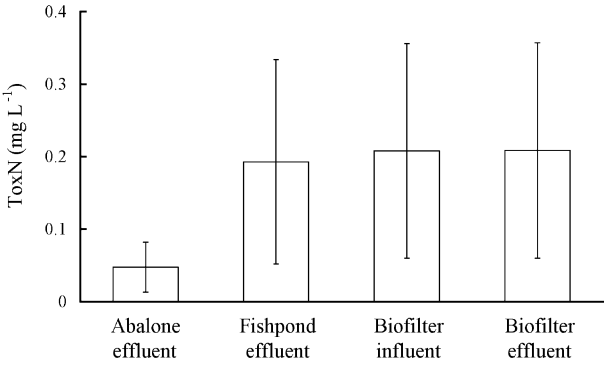


Fig. 3. Levels of ToxN as revealed by water analysis throughout the system. Averages of all sampling days (error bars: ± SD).

3.2. Diurnal patterns

A match of daily peaks in fish and seaweed metabolism was successfully achieved. Feeding the fish during the first half of the day synchronised the highest level of ammonia excretion with the fastest light-dependent nutrient uptake in the biofilter (Table 3). At 14:00 h, even though fishpond TAN concentrations were highest, TAN uptake efficiency of *U. lactuca* was also highest (96%; ± 3.1). At other sampling times, uptake efficiencies were lower, but nutrient concentrations in the fishpond effluent were also lower. Minimum uptake efficiency of 42% (± 13) at 02:00 h was nevertheless substantial. Uptake of *o*-phosphate followed a similar 24 h pattern (Table 3).

During the day, the pH of the recirculated water from the biofilter rose to almost pH 9 (Table 3). However, at the same time, fishpond pH only increased to pH 8. Oxygen levels were high throughout the system. During times of highest metabolism and respiratory oxygen demand in the fishpond, the recirculated water from the biofilter was super-saturated by up to 50% (an underestimate, as polarographic oxygen electrodes do not accurately measure DO in supersaturated solutions).

Levels of ammonia entering the system from the abalone tanks always remained below 0.07 mg l^{-1}. However, measured concentrations of *o*-phosphate there were rather high, at around 0.04 mg l^{-1} (Table 3). Generally, abiotic parameters in the influent from the abalone tanks fluctuated less than in the other units of the system.

3.3. Temperatures and the effect of the biofilter greenhouse

At night, outside air temperature reached levels as low as 5 °C. When the biofilter was covered with a greenhouse in early January, heat input to the fishpond via water recirculated from the biofilter increased (Fig. 4). A general heat loss was observed at night, reaching its maximum in the early morning hours, while the most heat was gained around 16:00 h (Fig. 5).

Table 3
Average abiotic parameters (± SD) in the fishpond and its two influents. Determined every 6 h (GH = greenhouse)

Time (h)		TAN (mg l^{-1})	PO_4 (mg l^{-1})	ToxN (mg l^{-1})	Water (pH)	DO (mg l^{-1})	Water temperature (°C)	
							December (no GH)	January (with GH)
02:00	abalone tanks	0.042 ± 0.03	0.037 ± 0.02	0.034 ± 0.02	8.26 ± 0.05	10.40 ± 0.76	21.0 ± 0.46	20.4 ± 0.53
	fishpond	0.570 ± 0.11	0.142 ± 0.07	0.202 ± 0.15	7.78 ± 0.07	10.70 ± 0.71	20.7 ± 0.50	20.2 ± 0.66
	biofilter recirculate	0.364 ± 0.11	0.140 ± 0.07	0.217 ± 0.15	7.80 ± 0.12	10.27 ± 0.57	17.7 ± 1.04	19.1 ± 0.65
08:00	abalone tanks	0.019 ± 0.02	0.051 ± 0.03	0.050 ± 0.03	8.24 ± 0.07	10.25 ± 0.86	20.7 ± 0.52	20.1 ± 0.65
	fishpond	0.530 ± 0.13	0.119 ± 0.05	0.182 ± 0.12	7.80 ± 0.09	10.23 ± 0.84	20.0 ± 0.59	19.2 ± 0.78
	biofilter recirculate	0.168 ± 0.11	0.087 ± 0.04	0.194 ± 0.14	8.17 ± 0.11	11.01 ± 1.13	16.6 ± 1.63	18.0 ± 0.84
14:00	abalone tanks	0.056 ± 0.06	0.043 ± 0.03	0.069 ± 0.06	8.27 ± 0.10	10.05 ± 0.78	21.8 ± 0.55	21.3 ± 0.41
	fishpond	0.794 ± 0.21	0.102 ± 0.06	0.184 ± 0.14	8.02 ± 0.12	9.91 ± 0.81	21.5 ± 0.65	21.9 ± 0.77
	biofilter recirculate	0.039 ± 0.03	0.062 ± 0.04	0.205 ± 0.15	8.88 ± 0.21	12.10 ± 1.32	20.9 ± 1.11	23.1 ± 0.58
20:00	abalone tanks	0.062 ± 0.04	0.036 ± 0.02	0.037 ± 0.02	8.24 ± 0.08	10.09 ± 1.10	21.4 ± 0.50	21.0 ± 0.47
	fishpond	0.724 ± 0.25	0.144 ± 0.09	0.202 ± 0.15	7.83 ± 0.14	10.64 ± 1.23	21.6 ± 0.61	21.7 ± 0.57
	biofilter recirculate	0.310 ± 0.17	0.116 ± 0.08	0.218 ± 0.16	7.96 ± 0.16	10.20 ± 1.06	19.1 ± 0.85	21.1 ± 0.72
24 h average	abalone tanks	0.045 ± 0.04	0.042 ± 0.02	0.047 ± 0.03	8.25 ± 0.07	10.20 ± 0.88	21.2 ± 0.51	20.7 ± 0.51
	fishpond	0.654 ± 0.18	0.127 ± 0.07	0.193 ± 0.14	7.86 ± 0.11	10.37 ± 0.90	21.0 ± 0.59	20.8 ± 0.70
	biofilter recirculate	0.220 ± 0.11	0.101 ± 0.06	0.208 ± 0.15	8.20 ± 0.15	10.90 ± 1.02	18.6 ± 1.16	20.3 ± 0.70

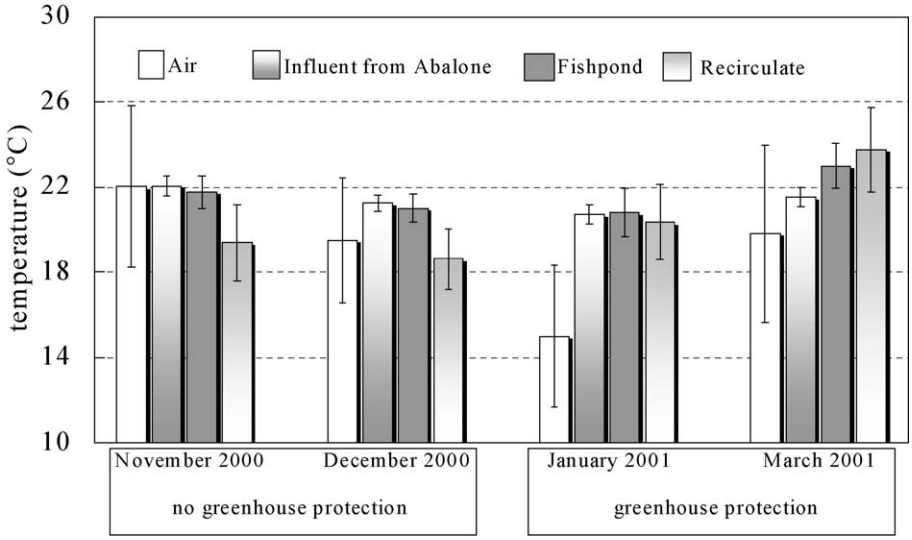


Fig. 4. Average monthly temperatures of air, fishpond and its two influents (recirculate = the water returning from the seaweed biofilter to the fishpond). During November and December, the biofilter was without a greenhouse and open to the environment (error bars: ± SD).

Compared to recirculated water, the 24 h temperature range of water entering the fishpond from the abalone tanks was narrow (Table 3). The average temperature of this water decreased with the progress of winter, before increasing again in March. With the filter covered by a greenhouse, temperature in the recirculated water was higher than in the water from the abalone tanks.

Air temperature inside the greenhouse was maintained above outside levels (Table 4) and wind-driven convection was reduced (personal observation). Correspondingly, and in contrast to the fishpond that was continuously covered with a greenhouse, Eq. (1) (heat changes independent of water flow) in the biofilter differs largely between times with and without a greenhouse (Fig. 5). In November, there was an average 24 h heat loss of 187 kcal m^{-2} h^{-1}. During the significantly colder month of January, the newly constructed greenhouse decreased this loss to only 46 kcal m^{-2} h^{-1}, while in March—roughly

Table 4
Average minima and maxima of air temperature inside and outside the biofilter greenhouse (± SD)

	Inside		Outside		Greenhouse effect	
	min	max	min	max	min (%)	max (%)
Air temperatures (°C)	13.7 ± 2.7	43.5 ± 4.7	10.1 ± 3.8	27.5 ± 5.6	35.6	58.0

Greenhouse effect = percentage increase of air temperature compared to outside levels.

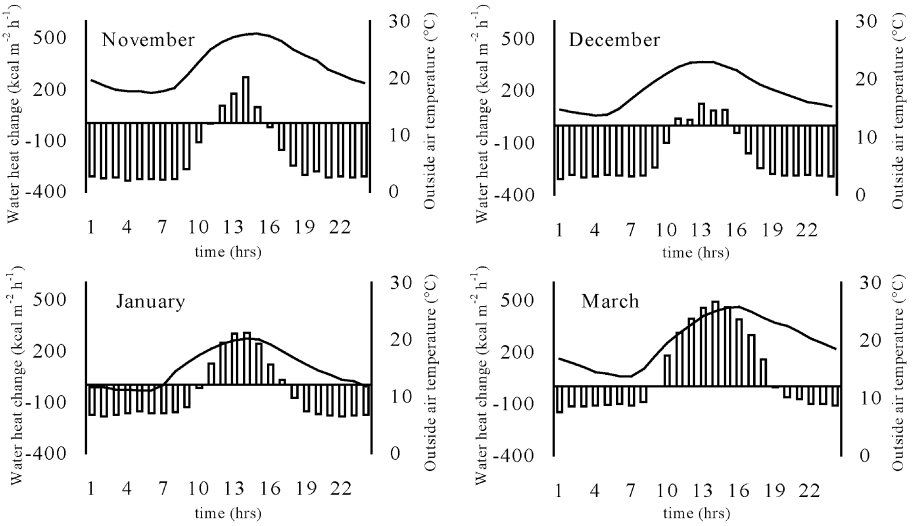


Fig. 5. Monthly averages of hourly changes in water heat due to fluxes other than heat import via water flow in the biofilter (Eq. (1)) (bars). Monthly averages of changes in outside air temperature are plotted for comparison (line). During January and March, the biofilter was covered with a greenhouse.

comparable to November or December (Fig. 4)—there was an average gain of 74 kcal m^{-2} h^{-1}.

4. Discussion

4.1. Balance between modules

Water recirculation through the seaweed biofilter was found to be useful with respect to levels of nutrients, dissolved oxygen, and CO_2. The peak synchronisation achieved between fish metabolic activity and seaweed photosynthesis stabilised water quality, which reduced the necessity for water quality control. During digestion and higher daytime activity, fish consume more oxygen, while their CO_2 excretion reduces the pH of their environment. At the same time, the seaweed produces oxygen and raises the pH by consuming CO_2. In a densely stocked intensive fishpond, where oxygen supersaturation is generally not a problem, the recirculated water provides the fish with an additional supply at times of highest oxygen demand.

Maximum levels of TAN, coinciding with the highest pH in the fishpond, never exceeded an all-time high of 1.3 mg l^{-1} and were always well below the lower limit of ammonia toxicity to the sea bream (2 mg l^{-1} at pH 8.2) (Bower and Bidwell, 1978; Wajsbrot et al., 1991). Maximal ammonia excretion by the fish coincided with highest ammonia removal and oxygen production in the biofilter, and recirculated water with a high pH and oxygen level contained very low levels of TAN. Thus, the recirculated water

from the biofilter moderated peaks in TAN excretion, oxygen consumption and CO_2 release in the fishpond.

On a daily average, the biofilter removed about 70% of available ammonia. In the presence of TAN, ToxN is not utilised by *U. lactuca* (Neori, 1996). Progressively higher ToxN concentrations that developed in the system with time therefore raised the fraction of the N-budget being lost in the effluents. Reduced N/P ratios of *U. lactuca* in the second period may be interpreted as the result of a certain N-limitation. Most probably, the growth of nitrifying bacterial biofilms on surfaces of the fishpond and in pipes leading to the biofilter produced this increase of ToxN. Since the biofilter was regularly cleaned during harvests, no significant nitrifying communities developed and no ToxN was formed on its walls. This result reconfirms that the establishment of large nitrifying communities can be prevented by occasionally scrubbing the water-covered surfaces (Dvir et al., 1999).

The discrepancy between the high concentrations of *o*-phosphate measured in the fishpond effluent and the lower values anticipated by published *o*-phosphate budgets is most likely explained by the relatively high level, introduced with incoming water from the abalone tanks (Table 3), alongside possible remineralisation of P from faeces and uneaten feed. As a result, the estimated uptake efficiency for P introduced with fish feed was double that of calculations from water analysis (Table 2).

The average seaweed yield obtained in the present winter study remained about 50% below summer yields as reported in Cohen and Neori (1991). It is therefore most likely that seaweed growth in the system studied here could increase during summer, suggesting that higher biofiltration rates could be expected in summer.

The production of high-protein *U. lactuca* can improve the economic viability of the integrated system. According to Shpigel et al. (2000), the abalone *H. discus hannai* fed with such seaweed grew with an FCR of 8.4 compared to over 25 if fed with low-protein *Ulva*. Therefore, using the concept of the novel three-stage biofilter, it may be possible to support the cultivation of more than twice as much abalone using the same seaweed production area.

4.2. Fish growth

Due to spawning triggered by shortened day length (Ben Tuvia, 1979), fish growth during the experimental period was lower than in other seasons. This effect can be avoided by artificial lengthening of the day with fluorescent light when day length starts decreasing significantly in September (Kissil et al., 2001). The reproductive patterns are also reflected in the increase of weight average SD values between the beginning and end of the experimental period. Standard deviation of final average fish weight (452 g) was increased to a very high ± 67, indicating that some fish had lost weight, while others had grown (Table 2). These results can be related to Kissil et al. (2001), who found that females of *S. aurata* lose weight during the reproductive period due to gonad production, while males continue to grow. Therefore, we feel safe in stating that the relatively slow growth of fish during the experiment was not related to water recirculation. It can also be ruled out that reduced fish growth was due to low temperatures. During the coldest period, the biofilter greenhouse maintained similar or even higher temperatures in the fishpond than when

outdoors (Fig. 4) and during all 5 months of recirculation, these values never dropped below 18 °C.

4.3. The greenhouse

Greenhouses are especially applicable to intensive desert aquaculture, where sharp diurnal fluctuations in air temperature can impact production. The biofilter greenhouse reduced nighttime heat loss from the water and improved heat gain during daylight hours by increasing ambient air temperature within the structure and excluding the cooling effect of convection. Consequently, and as expected from Ashkenazi et al. (2000), the overall temperature rose throughout the system. Although the yield of *U. lactuca* increased significantly under the greenhouse, it was not necessarily due to the higher temperature. Many factors, such as fluctuations in day length and light levels (including the shielding of UV radiation by the PVC foil of the greenhouse) and general plant physiology, were not determined and may have played a role in increasing the yield in the second period. With the biofilter greenhouse in place, the fish were expected to grow faster under higher temperatures (Lupatsch and Kissil, 1998), an effect annulled in this experiment by the onset of their reproductive period.

5. Conclusion

The proposed culture system for marine fish and high-quality seaweed allows expansion of mariculture without a concomitant increase in pollution, as opposed to sea cages or traditional flow-through ponds. A significant cut in water use and safer conditions in the fishpond are accompanied by less nutrient release into the environment. This may widen the scope for farm site selection near delicate marine areas and further away from the coast. It also reduces the introduction of pollutants, pathogens and fouling organisms to the mariculture system. The use of greenhouses allows the system to maintain a heat budget providing potentially better fish growth and biofilter performance. The conversion of nutrient pollution into useful, high-quality seaweed biomass can offset the costs of biofiltration.

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References

AOAC, 1980. In: Horwitz, W. (Ed.), Official Methods of Analysis of the AOAC, 13th ed. AOAC International, Arlington, VA, USA.

Ashkenazi, A., Mozes, N., Seginer, I., 2000. Heat budget model for predicting water temperature in outdoor intensive fish tanks under greenhouse cover. Proceedings of the Third International Conference on Recirculating Aquaculture, July 20–23, 2000. Roanoke, Virginia, USA, pp. 289–293.

Ben Tuvia, A., 1979. Studies of the population and fisheries of *Sparus aurata* in the Bardawil Lagoon, eastern Mediterranean. Investig. Pesq. 43, 43–67.

Bower, C., Bidwell, J., 1978. Ionization of ammonia in seawater: effects of temperature, pH and salinity. J. Fish. Res. Board Can. 35, 1012–1016.

Cohen, I., Neori, A., 1991. *Ulva lactuca* biofilters for marine fishpond effluents: I. Ammonia uptake kinetics and nitrogen content. Bot. Mar. 34, 475–482.

Dvir, O., van Rijn, J., Neori, A., 1999. Nitrogen transformations and factors leading to nitrite accumulation in a hypertrophic marine fish culture system. Mar. Ecol., Prog. Ser. 181, 97–106.

Kissil, G.Wm., Lupatsch, I., Elizur, A., Zohar, Y., 2001. Long photoperiod delayed spawning and increased somatic growth in gilthead seabream (*Sparus aurata*). Aquaculture 200, 363–379.

Krom, M.D., Neori, A., 1989. A total nutrient budget for an experimental intensive fishpond with circularly moving seawater. Aquaculture 80, 345–358.

Krom, M.D., Grayer, S., Davidson, A., 1985. An automated method of ammonia determination for use in mariculture. Aquaculture 44, 159–177.

Krom, M.D., Ellner, S., van Rijn, J., Neori, A., 1995. Nitrogen and phosphorus cycling and transformations in a prototype, 'non-polluting' integrated mariculture system, Eilat, Israel. Mar. Ecol., Prog. Ser. 118 (1–3), 25–36.

Lupatsch, I., Kissil, G.Wm., 1998. Predicting aquaculture waste from gilthead seabream (*Sparus aurata*) culture using a nutritional approach. Aquat. Living Resour. 11 (4), 265–268.

Muir, J.F., 1996. A systems approach to aquaculture and environmental management. In: Baird, J.D., Beveridge, M.C.M., Kelly, L.A., Muir, J.F. (Eds.), Aquaculture and Water Resource Management. Blackwell Science, UK, pp. 19–47.

Neori, A., 1996. The type of N-supply (ammonia or nitrate) determines the performance of seaweed biofilters integrated with intensive fish culture. Isr. J. Aquac.-Bamidgeh 48 (1), 19–27.

Neori, A., Shpigel, M., 1999. Using algae to treat effluents and feed invertebrates in sustainable integrated mariculture. World Aquac. Mag. 30 (2), 46–51.

Neori, A., Cohen, I., Gordin, H., 1991. *Ulva lactuca* biofilters for marine fishpond effluents: II. Growth rate, yield and C:N ratio. Bot. Mar. 34, 483–489.

Neori, A., Krom, M.D., Ellner, S.P., Boyd, C.E., Popper, D., Rabinovitch, R., Davidson, P.J., Dvir, O., Zuber, D., Ucko, M., Angel, D., Gordin, H., 1996. Seaweed biofilters as regulators of water quality in integrated fish–seaweed culture units. Aquaculture 141 (3–4), 183–199.

Neori, A., Shpigel, M., Ben-Ezra, D., 2000. A sustainable integrated system for culture of fish, seaweed and abalone. Aquaculture 186, 279–291.

Porter, C.B., Krom, M.D., Robbins, M.G., Brickell, L., Davidson, A., 1987. Ammonia excretion and total N budget for gilthead seabream (*Sparus aurata*) and its effect on water quality conditions. Aquaculture 66, 287–297.

Shpigel, M., Neori, A., Ben-Ezra, D., 1998. *Ulva lactuca* as food source for abalone in a land-based integrated system. J. Shellfish Res. 17 (1), 362–363.

Shpigel, M., Ragg, N.L.C., Lupatsch, I., Neori, A., 2000. Protein content determines the nutritional value of the seaweed *Ulva lactuca* for the abalone *Haliotis tuberculata*, *H. discus hannai* and *H. fulgens*. J. Shellfish Res. 19 (1), 534.

Solarzano, L., Sharp, J.H., 1980. Determination of total dissolved nitrogen in natural waters. Limnol. Oceanogr. 24 (4), 751–754.

Wajsbrot, N., Gasith, A., Krom, M.D., Popper, D.M., 1991. Acute toxicity of ammonia to juvenile gilthead seabream *Sparus aurata* under reduced oxygen levels. Aquaculture 92 (2–3), 277–288.

Wajsbrot, N., Gasith, A., Diamant, A., Popper, D.M., 1993. Chronic toxicity of ammonia to juvenile gilthead seabream *Sparus aurata* and related histopathological effects. J. Fish Biol. 42 (3), 321–328.

Ziemann, D.A., Walsh, W.A., Saphore, E.G., Fulton-Bennett, K., 1992. A survey of water quality characteristics of effluents from Hawaiian aquaculture facilities. J. World Aquac. Soc. 23, 180–191.

Zucker, D.A., Anderson, J.L., 1999. A dynamic, stochastic model of a land-based Summer Flounder *Paralichtys dentatus* aquaculture firm. J. World Aquac. Soc. 30, 219–235.