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Nutrients and fine particulate matter released from sea bass (*Dicentrarchus labrax*) farming

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Abstract – Mass budget of the sea bass (*Dicentrarchus labrax*) farming losses was examined. Experiments were carried out in April, September and November 2001 in tanks of 8.5 m^3 containing sea bass of 1, 31 and 53 g, respectively. Samples were taken at the input and discharge points of the tank at hourly intervals over 24 h. They were later analysed for nutrient concentration, as well as particulate organic carbon and nitrogen, chlorophyll *a* (Chl *a*) and total bacterial abundance. Despite the high water supply (exchange rate: 50% h^{-1}), NH_4 concentration was significantly higher in discharge water than in input water in all three experiments independent of fish size. The same was found to hold true for PO_4 for part of the day (April, November) or for the entire day (September). NO_3 and SiO_2 ions did not show any differences during April. Particulate organic carbon (POC) and nitrogen (PON) were higher in the discharge water in all seasons. Chl *a* concentration was higher in the discharge water during the entire day in September whereas bacteria presented higher counts in the output water only during part of the day in April. It was found that 5−7% of the nitrogen supplied is released into the water column as fine particulate material in the form of PON and 21−29% as NH_4. A small proportion (13−16%) of supplied phosphorus (P) was released as PO_4. The results provided in the present paper are useful in assessing environmental changes in water quality in the vicinity of fish farms in the Mediterranean.

Key words: Fish farm waste / Nutrients / Fine particulate material / Diel patterns / *Dicentrarchus labrax*

Résumé – Rejets aquacoles de nutriments et de matières particulaires d'une pisciculture de bar (*Dicentrarchus labrax*). Le budget des rejets d'une pisciculture du bar (*Dicentrarchus labrax*) a été examiné. Trois expériences ont été effectuées en avril, septembre et novembre 2001 dans des bassins de 8,5 m^3 contenant des alevins de bar de 1, 31 et 53 g respectivement. Des échantillons d'eau, pris à l'entrée et à la sortie d'eau toutes les heures, durant 24 h, ont été analysés pour mesurer les concentrations en nutriments, en chlorophylle *a*, et en carbone et azote organiques particulaires, ainsi que les bactéries. Malgré un fort taux de renouvellement de l'eau des bassins (taux d'échange : 50 % h^{-1}), la concentration en NH_4 a été significativement plus élevée en sortie, pour les trois expériences, c'est-à-dire indépendamment de la taille des alevins. Le même résultat a été trouvé pour les phosphates pour une partie de la journée (avril, novembre) ou pour la journée entière (septembre). En avril, aucune différence n'a été observée pour les concentrations en nitrates et silicates entre l'entrée et la sortie. Les concentrations de carbone organique particulaire (POC) et azote organique particulaire (PON) étaient plus élevées à la sortie d'eau pendant toutes les expériences. La concentration de chlorophylle *a* était plus élevée à la sortie d' eau pendant la journée entière en septembre et celle des bactéries seulement pendant une partie de la journée en avril. Les résultats de cette étude sont utiles pour évaluer les changements de la qualité de l'eau à proximité des piscicultures en Méditerranée.

1 Introduction

The recent expansion of sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*) cage aquaculture in the Mediterranean has caused concerns regarding widespread effects of aquaculture on the marine environment (Katranidis et al. 2003). Impacts of fish farming on the seabed are known both for salmonids (Gowen and Bradburry 1987; Weston 1990; Wildish et al. 2003; Pohle et al. 2001; Kemph et al. 2002) and non salmonid species (Karakassis et al. 2000). However, these impacts normally affect only the immediate vicinity of the farms whereas the release of nutrients and fine particulates can affect larger spatial scales, thereby affecting other users of the coastal zone (Machias et al. 2004, 2005; Pitta et al. 2005).

Field studies on water quality have failed to detect any significant changes in eutrophication-related variables (Beveridge 1996; Pitta et al. 1999; Soto and Norambuena 2004) in the immediate vicinity of the fish farms. However, mass balance

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Table 1. Experimental conditions.

Parameter	1st exp. (*)	2nd exp.(*)	3rd exp.(*)
Sampling date (2001)	March 31-April 1	Sept. 14–15	Nov. 9–10
Fish size (g)	1	31	53
Fish number	4000	1750	640
Tank volume (m^3)	8.5	8.5	8.5
Exchange rate (% h^{-1})	56	50	76
Temperature (°C)	18.9	23.9	20.4
Salinity	37.1	37.5	37.3
pH	8.15	8.20	8.14
Supplied food (kg)	950	620	340
Food type	INVE Epac Alpha 8/12	BIOMAR Ecostart No 5	Trouvit Europa Marine food

models published for several marine species showed that only a small fraction of the N and P provided through fish feeds is retrieved through harvesting in salmonids (Gowen and Bradbury 1987; Folke and Kautsky 1989; Holby and Hall 1991; Hall et al. 1992), sea bass (Dosdat et al. 1996; Kaushik 1998; Lemarié et al. 1998) and sea bream (Krom et al. 1985; Kaushik 1998; Lupatsch and Kissil 1998). Despite differences in the approach and experimental design of these studies, they have all shown that dissolved nutrients are a large proportion of the amount supplied through fish feed reaching ca 11–42% of P (typically ca 15%) and 31–80% of N (typically ca. 50%).

Although these nutrients are unlikely to have a large scale effect (Karakassis et al. 2005), the dissolved nutrients and fine particulate material have been considered the cause of changes in fouling communities in the vicinity of fish farms, effects on seagrass meadows (Holmer et al. 2003) as well as of changes in wild fish biomass and abundance at meso-scales (Machias et al. 2004, 2005). Fine particulate material has not been studied in detail in most mass balance data published although its fate in marine environments and its significance for heterotrophic food chains is potentially important when released in large quantities. Various sources could potentially contribute to this fine particulate material such as dust coming with fish feed, particles generated due to incomplete consumption of pellets by farmed fish, erosion of faecal material and bacteria associated with various biological processes in the fish farming system. It could be expected that some of these sources change with the age/weight of the farmed fish and consequently with changes in the size of feed pellets used.

The overall objective of the paper was to obtain quantitative estimates of the impact of fish farming on water quality that could be used for environmental impact assessment of cage farming. To this end we have used as a proxy a series of experiments in tanks which allow the quantification of the released wastes. The experiments involved measurement of total fine particulate matter, bacteria and dissolved nutrients released from sea bass at various stages of growth in fish tanks. Furthermore, the study was designed to take into account changes during diel cycle since it has been shown (Karakassis et al. 2001) that waste production by fish is not evenly distributed throughout the day and therefore it is likely that diel variability could result in overestimation or underestimation of waste production.

2 Materials and methods

2.1 Sampling design

Three sampling experiments were carried out in experimental tanks at the aquaculture station of the Hellenic Centre for Marine Research in Heraklion Harbor. Seawater samples were collected at the input and the output point of tanks containing a known quantity and total biomass of sea bass. The experiment was repeated for three different stages of development. Samples were collected at 1 hour intervals over a period of 24 h in all three experiments. A synopsis of the three experimental conditions is presented in Table 1. The first experiment was carried out on March 31st and April 1st 2001. There were 4000 fishes in the tank with a mean weight of 1 g. Water temperature and water supply were 18.9 °C and 4.8 $m^3\,h^{-1}$ respectively. The second experiment was carried out on September 14th and 15th 2001. There were 1750 fishes in the tank, all 202 days old with a mean weight of 31 g. The third experiment was carried out on November 9th and 10th 2001. There were 640 fishes in the tank, all 260 days old with a mean weight of 53 g. Fish were fed by means of an automatic feeder, which released food pellets in the tank periodically from 08:00 to 18:00. The flow rate was measured using a 20 L recipient and measuring the time taken to fill it with an electronic chronometer. The flow rate was measured 3 times during each experiment.

2.2 Chemical and biological analyses

Samples for the analysis of photosynthetic pigments were filtered through glass fiber filters (Whatman GF/F). Filters were extracted in 90% acetone for 24 h and fluorescence was measured by means of a Turner fluorometer according to the method of Yentsch and Menzel (1963). The filtrate was kept frozen at −20 °C and analysed for phosphates, silicates, nitrites and nitrates after Strickland and Parsons (1972) and for ammonia after Ivancic and Deggobis (1984). Fine particulate organic carbon (POC) and nitrogen (PON) were determined using a Perkin Elmer 2400 CHN Elemental Analyzer according to the procedure described by Hedges and Stern (1984). The term fine particulate was used to denote the particulate material that can be found in the water output i.e. the part of the material that does not directly sink on the bottom of the tanks.

Samples for counts of heterotrophic bacteria were preserved with borax-buffered formalin at a final concentration of 2%. Sub-samples of 5 mL were filtered on black Nuclepore filters (0.2 μmole L^{-1} pore size), stained with DAPI (Porter and Feig 1980), stored at −20 °C and counted by epifluorescence microscopy (Olympus BX-90) under UV light excitation.

2.3 Calculations

In order to construct a mass budget of the fish farm discharges the percent losses were calculated using the following equation:

$$Q_i = \left[\left(C_{i,\text{output}} - C_{i,\text{input}}\right)(\text{flux}t) / (C_{i,\text{food}}\ X)\right] 100$$

where Q_i: percent losses (%), $C_{i,\text{output}}$, $C_{i,\text{input}}$: g m^{-3} concentration of compound i in output and input respectively, flux in $m^3\,h^{-1}$, t: experiment time (h), $C_{i,\text{food}}$: concentration of element i in feed (%) and X: amount of feed (g).

2.4 Statistical analysis

Analysis of variance was used in order to compare concentrations of various variables between the input and the output of the tanks. In these analyses time was used as block in order to remove from the experimental error the variation related to the temporal evolution of variables, thereby increasing the precision of the analysis (Krebs 1989).

3 Results

During the experiment carried out in March (individual fish weight ±1 g), NH_4 concentrations at the output varied from 3.5 to 8 μmol L^{-1} with an average value of 5.7 μmol L^{-1}. Maximum concentrations were observed during feeding time, as well as a couple of hours after feeding. At the input, concentrations were considerably lower ranging from 0.4 to 1.5 μmol L^{-1}, with no significant temporal variation (Fig. 1) and an average value of 0.6 μmol L^{-1}. Similar variations were observed for phosphate concentrations. The output values were consistently higher than the input ones. Maximum was reached during feeding time. Concentrations were 0.6−1 and 0.4−0.5 μmol L^{-1} in the output and input respectively (Fig. 1).

For NO_3, NO_2 and SiO_2, no differences were observed at the input and output. Values at the input were 13−19, 0.03−0.2 and 2−5 μmol L^{-1} for NO_3, NO_2 and SiO_2, respectively. Values at the output were 12−19, 0.02−0.3 and 2−5 μmol L^{-1} for NO_3, NO_2 and SiO_2, respectively (Fig. 1).

Fine particulate carbon and nitrogen showed similar trends with ammonium and phosphates, with higher values at the output than at the input. Values for POC ranged from 40 to 120 μg L^{-1} at the input and 135−300 μg L^{-1} at the output. PON values were 6−22 at the input and 23−54 μg L^{-1} at the output (Fig. 1).

During the first half of the experiment, bacterial counts showed similar values at the input and output whereas in the second half, bacterial concentrations at the output were considerably higher. This occurred because the fish were fed antibiotics in the morning. At the input, counts ranged from 115 000 to 247 000 cells mL^{-1} and at the output from 150 000 to 300 000 cells mL^{-1} (Fig. 1).

During the September experiment in fish tanks containing 1750 individuals with a mean weight of 31 g, results were similar to the first experiment. Ammonium and phosphate concentrations were substantially higher at the output than at the input (Fig. 2). The concentration of ammonium in this experiment varied from 7 to 10 μmol L^{-1} for the output and 0.4 to 0.9 μmol L^{-1} for the input. For phosphates, the concentration during the September experiment varied from 0.5 to 1 μmol L^{-1} for the output and 0.4 to 0.5 μmol L^{-1} for the input (Fig. 2). Regarding Chl*a*, concentrations were constant and similar for the input and output, excepting three values from 19:00 to 23:00, where there was an abnormal increase in concentration at the output attributed to the detachment of fouling material from the tank. This peak also appeared in the POC and PON values (Fig. 2). Bacteria counts were consistently higher at the output during the entire experimental period (Fig. 2).

During the November experiment (mean fish weight: 53 g), ammonium and phosphate concentrations at the output varied from 2 to 4 and from 0.3 to 2 μmol L^{-1} respectively. The input concentration remained stable for both nutrients with an average value of 0.5 and 0.3 μmol L^{-1} for ammonium and phosphates respectively. Bacterial counts, POC and PON were consistently higher at the output (Fig. 3).

Despite the high volume of water supply (>50% renewal h^{-1}), results of the present study showed a clear enrichment of output water from fish tanks with POC, PON, ammonium, phosphates and bacteria (Table 2). Significant differences ($p < 0.01$) between input and output were observed for all fish weights, for POC, PON, NH_4 and bacteria. For PO_4, the same was observed with the exception of the 53 g fish where the significance level was lower ($p < 0.05$). On the other hand, no significant differences were observed for nitrates, nitrites and silicates (Table 2).

Percentages of losses calculated for all three experiments in this study are shown in Table 3. Carbon, nitrogen and phosphorus mass balances were calculated using the difference in concentration of input-output and the amount of feed supplied during each experiment. The highest proportions of nitrogen were released in the form of ammonium and fine particulate matter.

Discussion

Fish size was not critical factor for dissolved nitrogen losses.Percentage of losses of ammonium varied from 19−21% (fish size 1 g), 20−27% (fish size 31 g) and 25−28% (fish size 53 g) (Table 3). This observation are in consistence with other reported data (Ballestrazzi et al. 1994; Dosdat et al. 1996; Lemarié et al. 1998; Franco-Nava et al. 2004). Total ammonia nitrogen (TAN) production was found to be relative constant, 37% for 64−100 g $fish^{-1}$ (seabass) and 41% for 509−591 g $fish^{-1}$ (sea bass) (Franco-Nava et al. 2004).

Ammonia production was lower than the ones reported by other authors (Ballestrazzi et al. 1994; Dosdat et al. 1996). Lemarié et al. (1998) calculated a 30−58% loss in the form of ammonium for a series of fish sizes (25−325 g). Dosdat et al. (1996) observed higher values (56−70%) for 30 g sea bass.

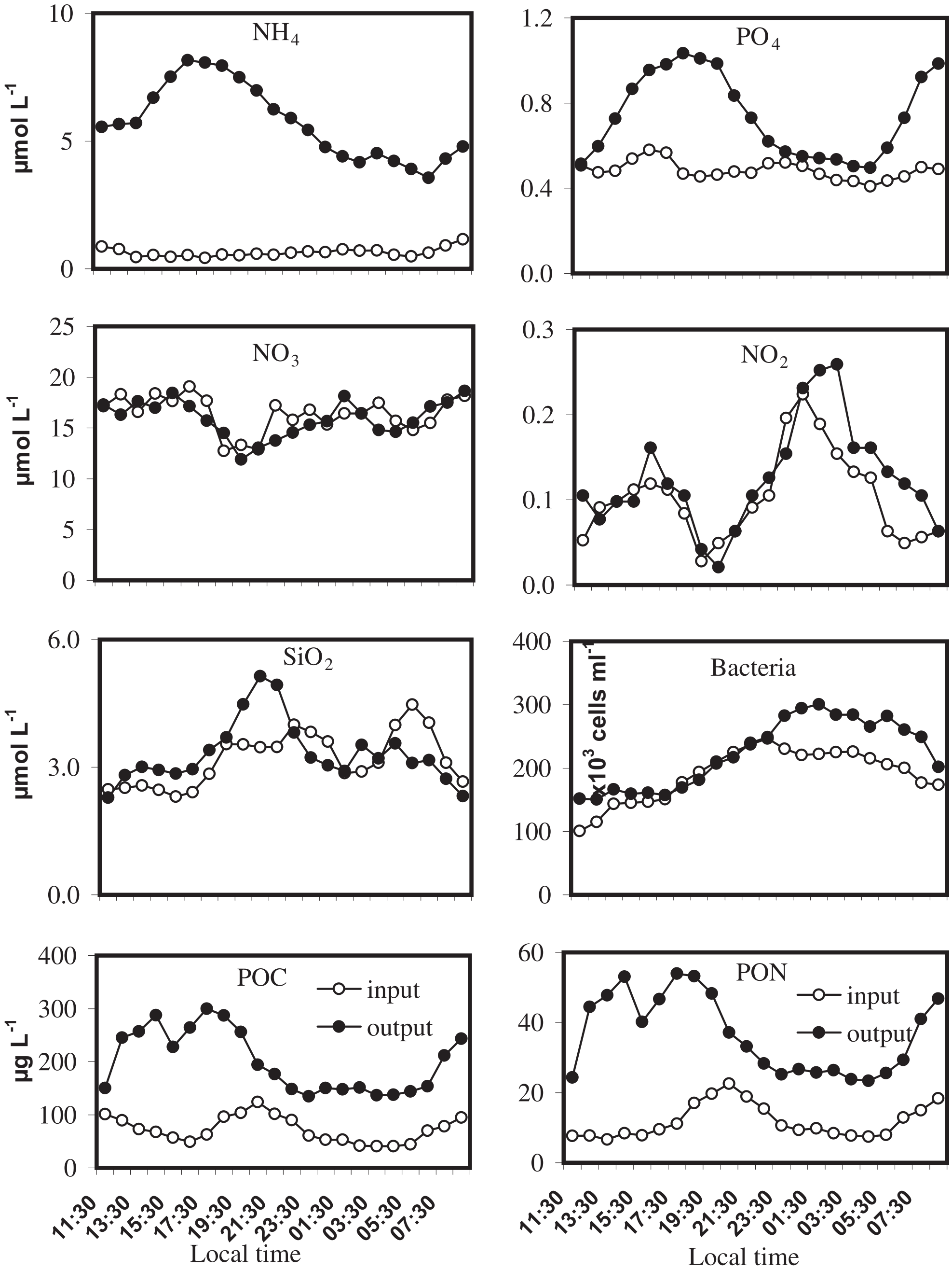

Fig. 1. Dynamics of nutrients (NH_4, PO_4, NO_3, NO_2, SiO_2), particulate organic carbon (POC) and nitrogen (PON) and bacterial counts for water input and output in tanks containing 1 g sea bass from March 31 to April 1, 2001.

This might be due to the fact that these values were calculated based on nitrogen intake, while values in the present study are based on nitrogen supplied. Furthermore, it has been extensively demonstrated that excretion of ammonium depends on the percentage of protein contained in the feed (Ballestrazzi et al. 1994). Fine particulate nitrogen percentages were 6−8% (fish size 1 g), 4−6% (fish size 31 g), and 6−7% (fish size 53 g).

Although urea was not taken into account in the present study, it is considered as a major nitrogen loss, excreted in dissolved form. Lupatsch and Kissil (1998) have published similar results. They found that 61% of the nitrogen supplied is excreted in dissolved form while a minimal percent (10%) is released as particulate faeces. This also holds true for trout aquaculture, where 48% of nitrogen is excreted dissolved and 23% in particulate form (uneaten feed and faeces) (Hall and Holby 1992).

The amount of phosphorus released in the form of phosphates varied from 13 to 16%, a relatively small percentage. No significant variations were observed with size. These results are compatible with Lupatsch and Kissil (1998), who estimated losses in dissolved form at 19%. The major fraction of phosphorus is released as particulate phosphorus and is estimated at 44% for sea bream (Lupatsch and Kissil 1998) and 50−57% for trout (Hall and Holby 1991).

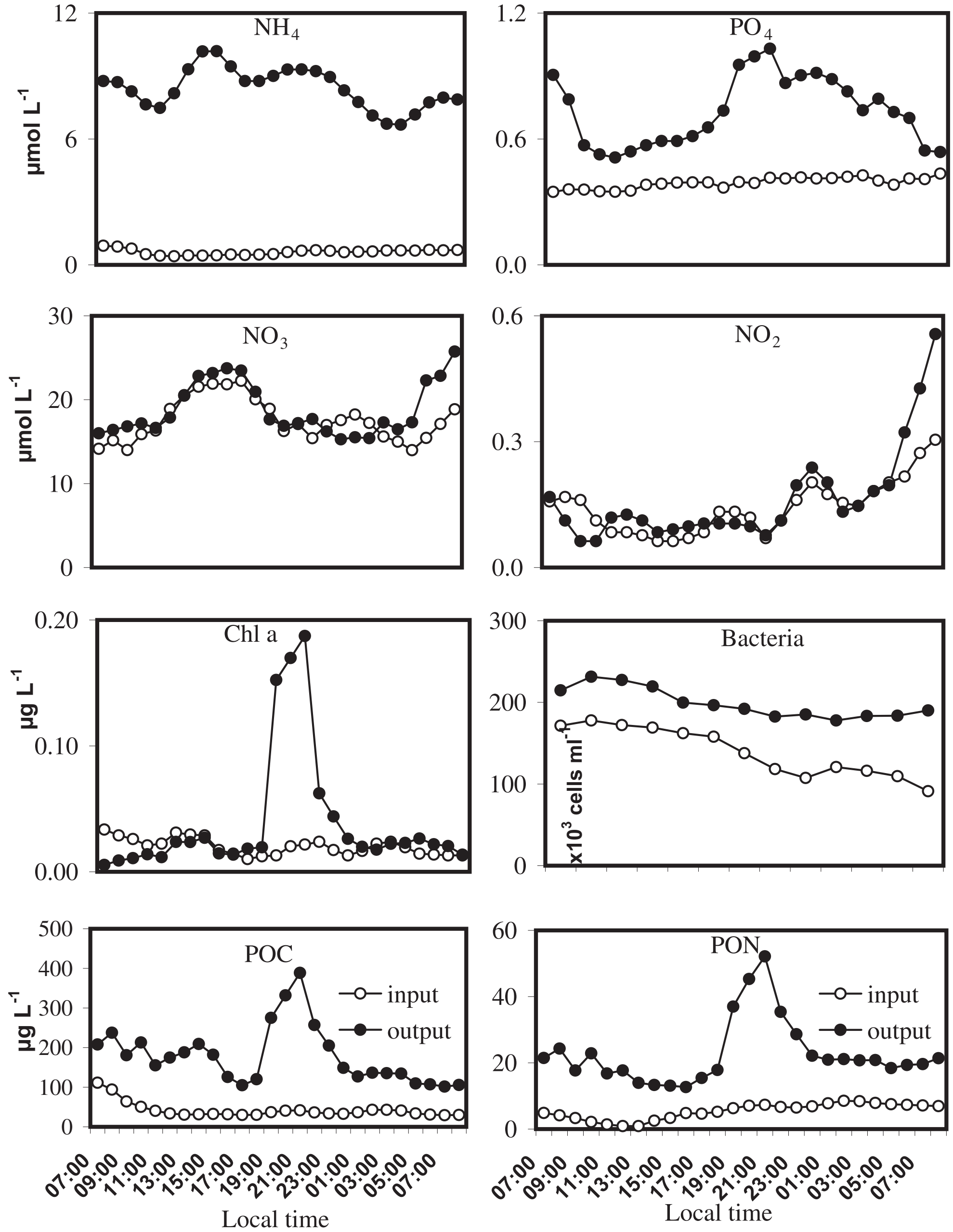

Fig. 2. Dynamics of nutrients (NH_4, PO_4, NO_3, NO_2), particulate organic carbon (POC) and nitrogen (PON), chlorophyll a, and bacterial counts for water input and output in tanks containing 31 g sea bass in September 14–19, 2001.

The fine particulate material comprises 5–7% of the nitrogen supplied through the fish feed. Despite the low quantity in relation to dissolved fractions, it is likely that this form of waste could play an important role in affecting the marine ecosystem in the vicinity of the fish farms:

- by favoring growth of suspension feeders in benthic communities of the seabed and hard substrates, and
- by affecting optical water properties (transparency) of the receiving water.

Some water column variables showed a diel change probably related to the feed-supply process which has also been found in field data in the vicinity of fish farms (Karakassis et al. 2001). This emphasizes the need for cautious design in monitoring water quality in fish farms, since timing could result in over- or underestimation of the discharge rates of ammonium and phosphate.

Bacterial abundance also seems to change by a factor of 2 in comparison to background values indicating intense microbial activity in the tanks. It is unclear what the exact reason is for this change in bacterial abundance. Most likely there are several reasons that explain growth of bacterial populations such as faecal discharges, exploitation of nutrients and mineralization of particulate wastes. However, there is a need to further investigate the nature of the bacterial types involved in

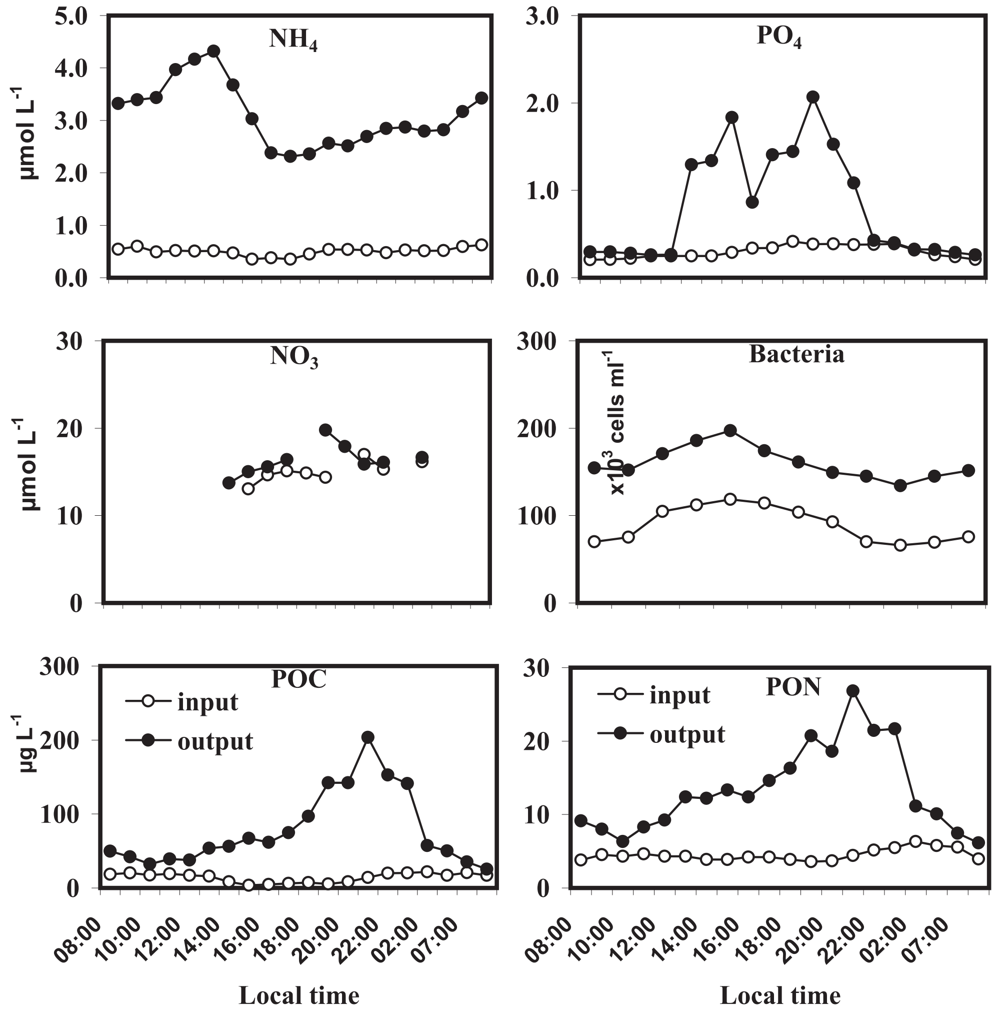

Fig. 3. Dynamics of nutrients (NH_4, PO_4, NO_3), particulate organic carbon (POC) and nitrogen (PON) and bacterial counts in water input and output in tanks containing 53 g sea bass over 24 h in November 9–10, 2001.

Table 2. Results of 1-way ANOVA (input vs. output) of water variables.

Fish weight (g)	1	31	53
		input vs. output	
POC	**	**	**
PON	**	**	**
NH_4	**	**	**
PO_4	**	**	*
Bact	**	**	**
NO_3	ns	ns	nc
NO_2	ns	ns	nc
SiO_2	ns	nc	nc

* $p < 0.05$, ** $p < 0.01$, nc: not counted, ns: non-significant.

Table 3. Nitrogen and phosphorus mass balance: percent losses*.

Fish weight (g)	PON (%)	NH_4 (%)	PO_4 (%)
1	7	21	13
31	5	29	16
53	7	27	13
Average	6	26	14

* All percentages expressed relative to feed input. Phosphorus percentage in the feed was 0.9–1%, Nitrogen was 6.7–7.2%.

order to arrive at safe conclusions regarding their role as indicators of a particular process in the system.

The range of fish size studied here was rather small (1–53 g) and therefore extrapolation to larger fish could be used with caution since larger feed pellets could behave differently in the water and faecal pellets sinking rates could also vary considerably with fish size. However, the estimates provided in the present paper are useful in assessing environmental changes in water quality in the vicinity of fish farms.

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