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Improvement of a system for treating land-based fish-farm effluents

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To improve the performance of a ponds system designed to treat land-based fish farm effluent and reduce nutrient release into the final water body, a series of modifications were implemented in 2003 to improve water circulation in the ponds and increase exchange between substrate and the water column. Special attention was dedicated to optimising the administration of fish-feed. The consequence was a slight improvement in environmental conditions, as indicated by comparison of levels of different variables (pH, dissolved oxygen, ammonium nitrogen and nitrates plus nitrites) between 2004 and 1999, before the modifications were made. In March 2004, two species of anemones, *Anthopleura ballii* and *Cereus pedunculatus*, were observed for the first time in the last two ponds. Estimates carried out between June and July 2004 showed a density of 100 and 200 individuals per square metre and a total biomass of about 700 kg wet weight. Although *A. ballii* is very tolerant of organic pollution, the presence of the two anemones could be interpreted as a sign of improved environmental conditions in the treatment system, as they represented energy flow towards higher levels of the food chain, suggesting that water recycling and rearing of saleable organisms in the treatment ponds are possibilities.

Keywords: Anthopleura ballii; Cereus pedunculatus; treatment pond; fish-farm wastewater treatment; water pollution remediation

1. Introduction

Aquaculture has become widespread enough to have significant impact on the environment and natural resources, producing various negative local environmental effects, such as eutrophication, oxygen depletion, modification of biodiversity and pollution of surrounding waters [1–6]. Fishfarm wastewaters contain high ammonium concentrations due to fish excretion through the gills, which may represent 75–85% of N loss [1]. Metabolic waste concentrations may reach high levels in tanks, limiting fish growth and survival [1,7]. Ammonia in undissociated form (NH₃) is one of the most toxic substances produced by intensive fish farms and has a high impact on aquatic communities when released into the environment [8]. The Water Research Centre (UK) suggests a safety limit of 0.021 mg l^{-1} (1.5 μ M) of non-ionised ammonia for fish life [1]. With an increase

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in pH, as often occurs during algal blooms, the non-ionised form of ammonia increases and may reach toxic levels.

Several systems can be used to reduce pollutant loads of intensive aquaculture effluents from land-based fish-farms. Sedimentation tanks or microscreens can reduce nutrient load of particulate and clarify wastewater [9]. Biological wastewater treatment by algal reactors and integrated systems has been tested over the past 30 years. Opportunistic macroalgae may reach suitable densities under controlled conditions, reducing dissolved nutrients [10–13]. However, these studies were mainly conducted in pilot plants and rarely on a real scale considering all the wastes produced by land-based fish farms having annual productions of several hundred tonnes [14–17].

The fish-farm of OPL s.r.l. at Nassa (Tuscany, Italy) is a rare example of a large-scale treatment system consisting of extensive ponds [15]. This need arose from the fact that the fish-farm in question discharges its wastewater into a lagoon, susceptible to eutrophication and with poor water turnover (Orbetello lagoon, Tyrrhenian Sea; Figure 1). This lagoon requires constant management to avoid dystrophic processes [18]. Treated urban wastewater and land-based fish-farm wastewater have been considered the main causes of lagoon hypertrophy [18,19]. Nutrient impact was estimated at 132.18 tonnes of N y⁻¹ and 6.66 tonnes of P y⁻¹ from the wastewaters of an urban treatment plant and four fish farms [18]. To reduce these inputs, the Nassa fish farm built a treatment plant for its effluent. The plant consisted of settling ponds for suspended particulate, where macroalgal blooms (mostly *Ulva laetevirens* Areschoug) occur in spring and summer [16].



Figure 1. Scheme of Nassa fish farm and its location. The 20 rearing tanks are shown above and the four treatment ponds below. The latter are indicated A, B, C and D, from right to left, from water inlet to outlet; the lines represent the plywood baffles introduced to increase mean residence time of water; the black dots (\bullet) in the first and last ponds indicate the positions of the multiparametric probes.

These blooms reduce wastewater nutrient loads in spring but are associated with sharp increases in pH and undissociated ammonia that can reach values dangerous to marine animals [20]. In summer, the algae decompose, reducing treatment efficiency [15,16]. These stress factors have prevented introduction of detritivorous/herbivorous species into the ponds to improve treatment efficiency [2,21,22].

Some of the problems afflicting efficiency of the treatment system are: (i) the high density of macroalgal development and logistic difficulties and costs associated with the harvesting and disposing of it; (ii) the relatively short residence time of water in the treatment ponds; and (iii) the relatively small area of the water-substrate interface. With regard to the latter, a greater exchange area increases the probability that nitrogenous solutes are nitrified and denitrified by bacteria and that a large portion of the nitrogen loses its toxicity and/or leaves the system [9]. Another problem of fish-farm management concerns the automatic feed dispensers. Although the frequency and amount dispensed by these devices can be regulated, they call for careful, continuous intervention of operators to check food demand that varies over the period of a day in relation to many factors. This means that feed distribution is not always optimised and that a fraction of the feed is wasted.

The aim of the present study was to assess the effects of some hydrodynamic and management modifications on the efficiency of the fish-farm effluent-treatment system, to improve the quality of wastewater from intensive aquaculture discharged into the Orbetello lagoon. Our initial hypothesis was that structural changes to the treatment ponds and optimisation of feed distribution would improve the quality of waste-water discharged from the plant.

2. Materials and methods

2.1. The study plant and modifications

In 1999–2000 the land-based fish farm at Nassa (Figure 1) produced about 100 tonnes y^{-1} of European sea bass (*Dicentrarchus labrax* L.) and gilt head sea bream (*Sparus aurata* L.) in 20 soft 450 m² PVC tanks (total volume 9000 m³) (Figure 1). The water used for the plant came from a channel joining Orbetello lagoon to the sea and flowed through the fish tanks and into a treatment system consisting of four ponds of the same dimensions (A, B, C, D) (Figure 1) arranged in series (overall 10451 m², mean depth 0.68 m, about 80% of the total rearing volume), and thence into a second channel between the lagoon and the sea. The water discharged into the channel was 139.5 1 s⁻¹ with a mean residence time (MRT) of 8 h [15].

In this study, MRT and the area of the water-substrate interface, where nitrification-denitrification processes occur [9], were increased. In July 2003, baffles of marine plywood were installed in the four ponds (Figure 1) so as to force the wastewater to circulate in all parts of the ponds and to increase the water-substrate interface. Furthermore, distribution of feed to the tanks was also optimised, reducing the quantity of solid and particulate matter going into the treatment system. An employee regulated the frequency of pellets, dispensed to fish by electrically-powered devices, according to demand.

However, it did not prove possible to solve the problem of the massive blooms of macroalgae for the following reasons: logistic difficulties, economic costs, and waste disposal legislation requiring disposal of harvested macroalgae in facilities for special categories of wastes.

In March 2004, several months after installation of the baffles in the treatment system, a great abundance of the anemones *Anthopleura ballii* Cocks 1951 and *Cereus pedunculatus* Pennant 1777 was observed in ponds C and D. Since the ponds were monitored continuously in the period 1998–2005, it can be stated with certainty that anemones were never observed before 2004. This does not mean that there were no specimens at all, but that they never reached the high densities

attained that year. It was therefore deemed important also to consider and quantify the development of anemones.

2.2. Abiotic and biotic parameters considered

As the preliminary basis for the study, the MRT of wastewater in the treatment system after installation of the baffles was calculated from pond size and volume, and rates of water output from the fish farm and the treatment system, were measured with a propeller type current meter (OTT mod. C2; Germany).

Temperature (t, °C), pH, salinity (S, psu) and dissolved oxygen (DO) (percentage saturation; DO%) were measured hourly from June to July 2004, using two multiparameter probes (HYDRO-LAB Datasonde 3; USA). These variables were measured at the inlet of pond A, receiving water directly from the fish farm, and at the outlet of pond D, where waters were finally discharged (Figure 1). Between April and July 2004, two water samples (three replicates) were taken monthly in ponds A and D. The samples were immediately filtered through a Whatman GF/F 0.70 μ m filter and stored at -20° C. In the laboratory, ammonium nitrogen (N-NH₄) and nitrates plus nitrites (N-NO_x) were analysed using a continuous-flow autoanalyser (BRAN + LUEBBE AA3; Germany). The results were compared with unpublished or only partially reported measurements obtained by the same methods between April and July 1999 [15]. This period was chosen because it was the only year prior to modification of the treatment system in which all the parameters necessary for comparison were available. Annual fish production and food conversion ratios (FCRs) in 1999 and 2004 were also compared.

Preliminary observations carried out in spring 2004 only revealed anemones in the last two ponds (C and D; Figure 1). Anemone counts were carried out in both ponds in June 2004, to quantify the phenomenon, and in pond D in July 2004, to compare spring and the critical summer period in the least affected pond furthest from the source of the disturbance. The surveys were carried out along parallel transects in a total of 40 regularly spaced stations. In each station, the counts were made *in situ* in an area of 900 cm², and the values were expressed per m². Thirty-five specimens collected at random and dabbed with blotting paper were used for wet weight determination.

2.3. Statistical analysis

Water nutrient data was analysed by three-way ANOVA to estimate the percentage of variance explained by annual and monthly temporal variability and by pond position. Normal distribution of data and homogeneity of variance were checked by the Kolmogorov-Smirnov and Levene tests, respectively, and when necessary, log(x + 1) transformation was used to normalise the distribution of data. Tukey's HSD test was used for *a posteriori* multiple comparison of means. Despite transformation, biotic parameters were still not normally distributed, so they were analysed by the non parametric Wilcoxon test to compare anemone abundance in two ponds (C and D) and in two months (June and July) in pond D. All statistical analysis was performed using the Statistica 7.0 software package.

Since chemico-physical data was not normally distributed after transformation, it was processed by non parametric multivariate analysis of variance (PERMANOVA) to test the responses of each variable to the factors *year*, *month* and *pond*, and to detect any significant effects of interactions between factors [23,24]. Significant terms detected by the test were investigated by *a posteriori* pairwise comparisons based on *t*-statistics. All statistical analysis was performed using the programme PERMANOVA+ for PRIMER (b18 version) [25].

Current-meter data was processed using BEAVER2 software. The results of MRT determinations were processed using a mathematical model [26].

3. Results

Despite an increase in annual fish production between 1999 [27] and 2004, food consumption and relative FCR showed a reduction (Table 1). In particular, FCR changed from 2.5 to 2.0.

After installation of the baffles in 2003, MRT was about 12 h, about 50% more than previously. Considering both sides, the baffles provided a water-substrate interface of about 500 m^2 , bringing the total exchange area of the system to about $11,000 \text{ m}^2$ (an increase of 4.78%). However, we do not know the actual area available before installing the baffles, since the water took the shortest path through the ponds, leaving many areas with little or no circulation. The baffles forced the water to circulate through all parts of the ponds.

Mean (\pm SD) DO (Table 2) varied significantly in relation to the factors *year* (Ye) and *pond* (Po); PERMANOVA revealed significant interaction effects (Table 3). Pairwise comparisons of Ye · Po indicated that mean DO in pond A was significantly higher in 2004 than in 1999 (p = 0.0001), suggesting less impact of effluent leaving the fish farm due to improved management. On the other hand, in pond D, there was no significant difference between the two years (Table 2). There were no significant differences between ponds A and D in 1999, whereas in 2004 DO was significantly higher in A than D (p = 0.0001). Temperature and salinity did not show significant variations.

Table 1. Food consumption (fc) in tonnes, annual fish production (fap) in tonnes, and food conversion ratio (FCR) for 1999 and 2004, in the Nassa fish farm.

	1999	2004
fc	211.01	181.58
fap	84.40	90.17
FCR	2.50	2.01

Table 2. Salinity (S, psu), temperature (t, $^{\circ}$ C), pH and percentage dissolved oxygen (DO%), in June and July 1999 and 2004, in the two extreme ponds (A, D) of the treatment system of the Nassa fish farm.

	А					D)	
	S	t	pН	DO %	S	t	pH	DO %
June 1999 July 1999 June 2004 July 2004	$\begin{array}{c} 37.3 \pm 0.4 \\ 37.4 \pm 0.3 \\ 35.5 \pm 0.3 \\ 38.3 \pm 0.4 \end{array}$	$\begin{array}{c} 24.2 \pm 0.8 \\ 26.9 \pm 0.6 \\ 24.0 \pm 0.9 \\ 26.5 \pm 0.8 \end{array}$	7.64 ± 0.25 7.80 ± 0.22 7.38 ± 0.21 7.38 ± 0.17	$68 \pm 38 \\ 57 \pm 30 \\ 97 \pm 59 \\ 95 \pm 54$	$\begin{array}{c} 37.7 \pm 0.5 \\ 39.1 \pm 0.4 \\ 37.2 \pm 0.3 \\ 38.6 \pm 0.3 \end{array}$	$\begin{array}{c} 24.6 \pm 0.4 \\ 27.3 \pm 0.6 \\ 23.8 \pm 0.7 \\ 26.3 \pm 0.7 \end{array}$	$\begin{array}{c} 7.71 \pm 0.19 \\ 7.67 \pm 0.23 \\ 7.52 \pm 0.43 \\ 7.26 \pm 0.45 \end{array}$	$65 \pm 52 \\ 58 \pm 43 \\ 59 \pm 36 \\ 59 \pm 47$

Table 3. Results of PERMANOVA analysis of dissolved oxygen (DO) and pH.

	df	D	0	р	pH	
Source		pseudo-F	P (perm)	pseudo-F	P (perm)	
Ye	1	12.3	0.0003	131.0	0.0001	
Мо	1	1.32	0.257	1.40	0.2338	
Ро	1	18.5	0.0001	0.1580	0.6944	
Ye · Mo	1	0.8649	0.3522	11.4	0.0007	
Ye · Po	1	16.9	0.0001	0.3824	0.5397	
Mo · Po	1	0.1283	0.7193	16.4	0.0002	
$Ye \cdot Mo \cdot Po$	1	0.0119	0.9100	0.2130	0.6424	
Res.	424					
Total	421					

Mean (\pm SD) pH (Table 2) was significantly affected by interaction effects Ye · month (Mo) and Mo · Po (Table 3). Pairwise comparison of Ye · Mo showed significantly higher pH in 1999 (June and July) than in 2004 (p = 0.0001). In A and D, pH was always low with respect to that of input seawater (8.26 ± 0.21). In June, pH was significantly lower in A than D (p = 0.0157); though this was on the contrary in July (p = 0.0009; pairwise comparison of Mo · Po).

Mean $(\pm$ SD) N-NH₄ (Table 4) varied significantly in relation to Ye, Mo and Po, and ANOVA detected significant effects for the interaction term (Table 5). The post hoc test applied to Ye · Mo · Po indicated that ammonium concentrations were significantly lower in April 2004 than 1999 in ponds A and D (p = 0.0002); the same was observed in July in pond D (p = 0.0044), while concentrations remained unchanged in A. In 1999, N-NH₄ in pond A was significantly higher in April than in May (p = 0.0017), June (p = 0.0003) and July (p = 0.0003); in pond D, N-NH₄ decreased in June (p = 0.0348) with respect to April, increasing again in July (p = 0.0033). On the other hand, in 2004 N-NH₄ was significantly lower in April than in May (p = 0.0259 in A; p = 0.0002 in D), June (p = 0.0340 in A; p = 0.0007 in D) and July (p = 0.0003 in A; p = 0.0003 in D). Ammonium was significantly higher in A than in D in April and June 1999 (p = 0.0002; p = 0.0022) and in April and July 2004 (p = 0.0164, p = 0.0220), but overall the percentage difference between input and output ((D – A) · 100/A) was -18.58 ± 14.99 in 1999 and -26.08 ± 13.18 in 2004 (Table 3).

Mean (\pm SD) N-NO_x concentrations (Table 6) were significantly affected by the interaction factor Ye · Mo · Po (Table 5), and post hoc comparisons showed that they were significantly lower in April 1999 than in April 2004 in D (p = 0.0333), while remaining unchanged in A (Table 6). Although input was similar in the two years, in 2004 this variable increased through the treatment ponds, being statistically significant in April. N-NO_x increased significantly from A to D in June and July 1999 (p = 0.0396; p = 0.0049) and in April, May and June 2004 (p = 0.0048; p = 0.0384; p = 0.0004). The treatment system ensured greater N-NO_x concentrations at the

Table 4. Mean (\pm SD) N-NH₄ and percentage difference (d% = (D - A) \cdot 100/A) between ponds A and D of the treatment system of the Nassa fish farm, in the period April–July 1999 and 2004.

A 1999D 1999d% 1999A 2004D 2004d% 2004April 80.40 ± 2.20 55.28 ± 4.51 -31.09 ± 8.30 35.00 ± 5.70 19.75 ± 6.33 -43.53 ± 4.53 May 56.70 ± 7.50 49.9 ± 3.00 -11.89 ± 8.10 49.58 ± 3.80 42.06 ± 4.21 -14.93 ± 3.53 June 59.20 ± 4.86 41.14 ± 4.22 -30.52 ± 10.29 49.17 ± 4.27 39.42 ± 2.56 -19.98 ± 1.55 July 59.25 ± 6.20 58.65 ± 6.11 -0.81 ± 2.56 56.38 ± 5.23 41.55 ± 3.64 -25.88 ± 9.55 Total 63.89 ± 10.69 51.24 ± 8.28 -18.58 ± 14.99 47.53 ± 8.78 35.69 ± 10.31 -26.08 ± 13.55							
April 80.40 ± 2.20 55.28 ± 4.51 -31.09 ± 8.30 35.00 ± 5.70 19.75 ± 6.33 -43.53 ± 4.51 May 56.70 ± 7.50 49.9 ± 3.00 -11.89 ± 8.10 49.58 ± 3.80 42.06 ± 4.21 -14.93 ± 3.53 June 59.20 ± 4.86 41.14 ± 4.22 -30.52 ± 10.29 49.17 ± 4.27 39.42 ± 2.56 -19.98 ± 112 July 59.25 ± 6.20 58.65 ± 6.11 -0.81 ± 2.56 56.38 ± 5.23 41.55 ± 3.64 -25.88 ± 9.52 Total 63.89 ± 10.69 51.24 ± 8.28 -18.58 ± 14.99 47.53 ± 8.78 35.69 ± 10.31 -26.08 ± 12.56		A 1999	D 1999	d% 1999	A 2004	D 2004	d% 2004
Total 63.89 ± 10.69 51.24 ± 8.28 -18.58 ± 14.99 47.53 ± 8.78 35.69 ± 10.31 $-26.08 \pm 13.24 \pm 10.24 $	April May June July	$\begin{array}{c} 80.40 \pm 2.20 \\ 56.70 \pm 7.50 \\ 59.20 \pm 4.86 \\ 59.25 \pm 6.20 \end{array}$	$55.28 \pm 4.51 \\ 49.9 \pm 3.00 \\ 41.14 \pm 4.22 \\ 58.65 \pm 6.11$	$\begin{array}{c} -31.09\pm8.30\\ -11.89\pm8.10\\ -30.52\pm10.29\\ -0.81\pm2.56\end{array}$	$\begin{array}{c} 35.00 \pm 5.70 \\ 49.58 \pm 3.80 \\ 49.17 \pm 4.27 \\ 56.38 \pm 5.23 \end{array}$	$\begin{array}{c} 19.75 \pm 6.33 \\ 42.06 \pm 4.21 \\ 39.42 \pm 2.56 \\ 41.55 \pm 3.64 \end{array}$	$\begin{array}{c} -43.53 \pm 4.66 \\ -14.93 \pm 3.76 \\ -19.98 \pm 11.02 \\ -25.88 \pm 9.96 \end{array}$
	Fotal	63.89 ± 10.69	51.24 ± 8.28	-18.58 ± 14.99	47.53 ± 8.78	35.69 ± 10.31	-26.08 ± 13.18

Table 5. Results of three-way ANOVA for ammonium $(N-NH_4^+)$ and oxidised nitrogen $(N-NO_x)$. Sources of variability: Year (Ye), Month (Mo), Pond (Po).

	df	N-N	M_4^+	N-NO _x	
Source		F	Р	F	Р
Ye	1	151.7	0.0000	0.134	0.7167
Мо	3	5.7	0.0031	1.553	0.2198
Ро	1	89.3	0.0000	90.6	0.0000
Ye · Mo	3	40.2	0.0000	5.1	0.0052
Ye · Po	1	0.0978	0.7565	4.5	0.0420
Mo · Po	3	5.6	0.0034	2.0	0.1301
$Ye \cdot Mo \cdot Po$	3	4.6	0.0091	3.6	0.0247
Res.	32				
Total	47				

	A99	D99	d% 1999 %	A04	D04	d% 2004 %
April	6.51 ± 1.03	8.20 ± 3.45	31.58 ± 62.36	6.53 ± 0.97	19.09 ± 1.28	194.82 ± 29.91
May	8.63 ± 1.11	12.38 ± 7.23	53.21 ± 103.21	7.75 ± 1.53	18.55 ± 2.13	142.47 ± 24.40
June	8.96 ± 1.89	22.59 ± 9.56	172.45 ± 151.94	4.64 ± 1.13	17.81 ± 3.45	286.83 ± 21.05
July	6.12 ± 1.77	18.18 ± 5.12	201.03 ± 69.53	7.28 ± 2.26	12.70 ± 4.49	72.80 ± 8.85
Total	7.56 ± 1.83	15.34 ± 7.90	114.57 ± 116.52	6.55 ± 1.82	17.04 ± 3.74	174.23 ± 83.79

Table 6. Mean (\pm SD) N-NO_x and percentage difference (d% = (D - A) \cdot 100/A) between ponds A and D of the Nassa fish farm, in the period April–July 1999 and 2004.

Table 7. Mean density (\pm SD; ind. m⁻²) of *Cereus pedunculatus* and *Anthopleura ballii* in Nassa fish farm treatment ponds C and D during June and July 2004.

	С	D	
June 2004 July 2004	40.3 ± 38.7	85.5 ± 118.2 73.4 ± 97.1	

outlet than at the inlet in both years, and the overall percentage difference between input and output $((D - A) \cdot 100/A)$ was greater in 2004 (Table 5).

Mean density (\pm SD) of anemones (Table 7) in June was lower in pond C than in D, whereas in D it dropped by 14% (not significant) in July with respect to June (Wilcoxon Tests, Z = -1.754, p = 0.079, Z = -0.214, p = 0.830, respectively). Mean individual wet weight (n = 35) was 2.45 ± 1.38 g. All randomly collected specimens turned out to be *C. pedunculatus*. The estimated 214,348 individuals in D (2507 m²) in June and 184,064 individuals in July would therefore weigh 525 kg and 451 kg, respectively. The estimated 93,496 individuals in C (2320 m²) in June therefore had a total weight of 229 kg.

4. Discussion

Greater attention paid to distribution enabled a reduction in the quantity of feed used, certainly helping to improve water quality at the outlet and repaying the cost of increased labour. However, although this was a good result, a FCR of 2.0 is still too high, and environmental practices must be improved by the fish farm management to reduce waste production.

Management of feed distribution was why DO was significantly higher in A than D in 2004, although fish biomass increased between 1999 and 2004. Respiration increased through the ponds, presumably due to high bacterial activity caused by accumulation of organic matter in sediment over the years [16].

Increased fish production and accumulation of organic matter had the better of pH that was lower in 2004 than 1999. Lower spring pH in pond A than D, and the opposite in summer, could be due to intense feeding and growth of fish and to a reduced demand for feed due to higher temperatures, respectively. On the other hand, in pond D, photosynthesis of the plant mass prevailed until June, followed by a prevalence of respiration when temperatures increased.

The lower N-NH₄ input into the treatment ponds in 2004 in the period of intense feeding may have been due to less wasted feed and increased oxygenation of the rearing tanks. In 2004, seasonal effects prevailed and N-NH₄ increased with increasing temperature and fish growth, and this was reflected in the treatment ponds, where respiration prevailed in the warm season. In 1999, the picture may have been confounded by an excess of feed. However, the treatment improved this variable in the two years studied, but the 26% reduction in N-NH₄ input (from

 $63.9 \pm 11.1 \,\mu\text{M}$ in 1999 to $47.5 \pm 9.0 \,\mu\text{M}$ in 2004), probably due to optimised administration of feed, and installation of the baffles improved efficiency in 2004 (Table 4).

In the period April–June 2004, N-NO_x increased significantly between A and D, and N-NH₄ remained unchanged in A with respect to 1999, despite production of a greater biomass of fish. Although this condition did not always occur and was confounded by intense respiratory activity in hot weather due to proliferation of algae in the treatment ponds, the results sustain the hypothesis that baffles increase nitrification by increasing the surface area available to nitrifying bacteria, even if the process was less efficient in the hottest month, probably due to collapse of the macroalgal mass and associated hypoxia.

A. ballii colonises substrates such as sand, mud and rock, and hosts symbiotic algae (dinoflagellata: zooaxanthellae) in its tissues, though its green colour is due to typical cnidaria proteins. Observations have shown that A. ballii only feeds on dead organic matter, whereas C. pedunculatus also needs to feed on live animals; under such environmental conditions, A. ballii can be expected to thrive before C. pedunculatus (A. Horton, pers. com.). A. ballii had never previously been observed, even in the Orbetello lagoon, whereas C. pedunculatus was known to exist [28]. The latter species is described as very tolerant to organic pollution and eutrophic stress; its mode of reproduction (sexual vs. asexual) is known to be affected by pollution: asexual reproduction in the form of parthenogenesis is said to occur in populations in the high intertidal zone, where the water is heavily polluted and organisms are one-half or one-third the size of those in subtidal populations, which reproduce sexually and are oviparous [28]. It colonises submerged hard bottoms close to the town of Orbetello in the centre of the lagoon, far from tidal flow from the sea-outlets.

In the treatment ponds, *A. ballii* was seldom observed submerged in the sandy-muddy bottom but mainly out of the sediment, adhering to hard substrates such as worm concretions, pebbles, shells and macroalgal thalli. *C. pedunculatus* was observed submerged in the sandy-muddy bottom but always adhering to hard substrates (especially pebbles and shells).

In June, development of the anemone biomass of about 754 kg in ponds C and D, having overall area of 4827 m² and volume of 3282 m³, was a good result, and could have triggered positive feedback, since these species have high water-filtration capacity and reduce suspended organic load together with sediment oxygen demand. The anemone biomass may have been sustained by the high (not quantified) biomass of crustaceans, which presumably increased for the same reasons. A similar species, *Actinia equina* [30], has been shown to require much food at sea temperatures typical of the eastern Mediterranean, suggesting that the relatively high temperature in the Nassa fish-farm treatment pond (Table 2) is not only a problem for this species but could determine a high metabolic rate and therefore a high feeding rate. The (non significant) decline in their development in D could be related to the decrease in feed. According to various authors [29,31,32], *C. pedunculatus* is tolerant of organic pollution and periodic extreme conditions in lagoon environments. Not much information is available about adaptation of *A. ballii* to lagoon or disturbed environments such as treatment ponds, though it is known to have a wide distribution, from temperate to tropical climates [33]. However, this species is very tolerant of organic pollution and its feeding habits suggest that it is more likely to thrive than *C. pedunculatus*.

5. Conclusion

The sudden appearance of anemones in 2004 could therefore be related to the slight improvement in environmental quality in treatment ponds C and D, with respect to environmental conditions in previous years, due to more rational food administration in the rearing tanks and installation of baffles in the treatment ponds.

Indeed, the appearance of anemones aroused much interest, since it suggested that the plant modifications had finally made it possible to introduce organisms into the treatment ponds to use energy available in the wastewater. The wastewater of fish farms that produce hundreds of tons of fish per year is difficult to treat at full scale, especially in limited treatment areas. Though the problem of managing algal proliferation remains, large treatment areas mean it is easier to mitigate problems and transfer energy contained in effluent to higher levels in the food chain. An improved treatment environment could enable a primary consumer of commercial interest to be introduced into the ponds, thus partially compensating increased management costs. Reduction of wastewater toxicity could enable water to be recycled to the rearing tanks, an important step for land-based fish-farms that use wells to exploit marine water-tables. If the presence of anemones is persistent, it may be a preliminary result towards a more rational management of fish farm wastewater.

These findings can be considered a contribution to the scientific discussion on how to improve aquaculture management in order to prevent or reduce pollution of natural waters by effluent [33]. The measures adopted should encourage use of further environmental best practices in fish farms with biological treatment of tank effluent, for example installation of sediment aerators to prevent onset of anaerobic processes, resolution of problems associated with removal of excessive macroalgal production before it dies in summer, reclamation (draining and mixing) of the first sediment layers to re-mineralise organic matter accumulating in the sediment ponds over the years, and installing further baffles to increase the available water-substrate interface.

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