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RESEARCH ARTICLE

Assessing changes in the toxicity of effluents from intensive marine fish farms over time by using a battery of bioassays

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Abstract

Although intensive marine fish farming is often assumed to be eco-friendly, the associated activity can lead to chronic exposure of marine organisms to potentially toxic discharges. Moreover, despite the increasing popularity of integrated multi-trophic aquaculture (IMTA), studies of the effects of fish farm effluents are almost non-existent. In the present study, the changes in the toxic potential of effluents from five land-based marine fish farms in NW Spain subjected for different lengths of time to a biodegradation procedure (for 0, 48, 120, and 240 h) were assessed in a battery of bioassays including organisms from different trophic levels (*Vibrio fischeri*, *Isochrysis galbana*, and *Paracentrotus lividus*). The results of the bioassays at the different times were then considered together with farm water flow in the Potential Ecotoxic Effects Probe (PEEP) index. Despite the high volumes of effluents discharged, the generally low toxicity of the effluents hinders assessment of potentially toxic effects. However, dose–response curves and statistical analysis demonstrated the existence of toxic effects during the first five days of the biodegradation procedure, especially immediately after sampling. The proposed modification of the PEEP index better reflects the changes in toxicity over time.

Keywords Mariculture · Toxicity persistence · Battery of bioassays · Bacterial luminescence · Microalgae growth · Larval development · DBPs · Disinfectants

Introduction

Fish play a fundamental role in human nutrition, and the growing demand from the world's population has led to the gradual depletion of marine resources (FAO 2016). Aquaculture has emerged as a possible solution to overfishing; however, to be sustainable, it must be respectful of the environment on which it depends directly (Carballeira et al. 2012a). Spain is the largest aquaculture producer in Europe and is a pioneer and top producer of turbot (*Scophthalmus maximus* L. 1758) in intensive marine land-based facilities (MAGRAMA 2016). Intensive pisciculture involves the management of high densities of fish and requires exhaustive control of culture conditions. This is mainly obtained by the application of chemical products to prevent the appearance and propagation of diseases. However, the effluents discharged to the aquatic environment mainly comprise the metabolic waste products of fish (Tello et al. 2010). Traditional flow-through systems, in which the water is circulated through tanks before being returned to the environment without being treated, are the most common type of system used in turbot farming in Spain.

Studies of the potential impact of waste discharges from aquaculture have traditionally been conducted by analysis of the physical–chemical properties of sewage. However, studies of the potential toxicity of waste discharges are scarce, and few bioassays have been used to determine the effects on affected biota and the potential toxicity of the waste (Carballeira et al. 2011, 2012a, b, c, d).

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The discharges from land-based aquaculture are generally of low toxicity because the effluents basically consist of the water in which fish are grown plus highly diluted chemicals. Few laboratory bioassays are sensitive enough to detect any effects on biota, even when early life stages or lower organisms are used. However, indices that take into account the large volumes discharged indicate very high levels of potential toxicity (Carballeira et al. 2012b). The *Potential Ecotoxic Effects Probe* (PEEP) is a useful index for assessing the impacts of pisciculture effluents, but it does not take into account changes in toxicity as a result of biodegradation processes (Costan et al. 1993; Carballeira et al. 2012b).

Many chemicals present in aquaculture discharges (e.g. ammonia and antibiotics) do not persist in the marine environment and are rapidly dispersed (Pitta et al. 2006). Nevertheless, considering the secrecy surrounding the chemicals used in the industry and the lack of previous toxicity persistence studies (Sapkota et al. 2008), it is possible that the effluents may contain some highly persistent chemicals, such as antifouling agents and pesticides.

The objective of the present study was to assess the changes in the toxicity of discharges from land-based fish farms over time by using a battery of bioassays including three marine organisms belonging to different trophic levels: *Vibrio fischeri* (bacterium), *Isochrysis galbana* (microalga), and larvae of *Paracentrotus lividus* (sea urchin). Ecotoxicological parameters and the PEEP index were used to express the potential toxicity, taking into account the emission flow rate and the persistence of toxicity.

Material and methods

Effluent sampling and the biodegradation procedure

Effluent samples were collected in high-density polyethylene bottles, between November (2015) and July (2016), from five land-based turbot farms in Galicia (NW Spain; Fig. 1). All farms use traditional flow-through aquaculture systems and similar management, as indicated by data provided by the regional government of Galicia during the 6-year sampling period (Carballeira et al. 2012b). The samples were transported to the laboratory in cool containers, to minimize oxidation of ammonia, and were immediately tested in the bioassays.

In parallel to the bioassays, the effluent samples were subjected to a biodegradation procedure based on commonly used protocols (Costan et al. 1993), but adapted to the particular conditions of the study area and of the samples. The effluent samples were maintained at 15 °C (in a cool chamber) for 240 h, with a high level of oxygenation, in darkness. The usual protocols (designed for freshwater effluents) require the addition of inorganic salt solutions; however, we did not include this step, as seawater already contains salts. In addition, we added clean seawater (see below) to the samples instead of adding a commercial bacterial inoculum. The clean seawater was collected from the same sites as the specimens of *P. lividus*, close to farm V, but far enough to be a clean site.

Physico-chemical characterization

Levels of ammonia, nitrites, and nitrates in water samples were determined by the Nessler, Griess-Ilosvay, and ultraviolet methods, respectively (Hach Company 1997; APHA 2012).

Electrical conductivity, dissolved oxygen, and turbidity were recorded at the beginning of the experiment with respectively a conductimeter (XS COND7), an oximeter (WTW Oxi320), and a nephelometer (Lutron TU-2016). Salinity and pH were determined by use of a multiparameter water quality meter (Hanna HI 9828).

Biological Oxygen Demand after 5 days (BOD_5) was determined with the Oxitop Determination System (WTW) following standard methods UNE-EN 1899 and UNE-EN 1899-2.

Toxicity bioassays

Bioassays were performed immediately after effluent sampling (0 h), and after 48, 120, and 240 h. In each test, organisms were exposed to six different dilutions (volume of effluent:volume of clean sea water) of each of the effluents from the fish farms: 0:1 (control), 1:20 (5%), 1:10 (10%), 1:4 (25%), 1:2 (50%), 3:4 (75%), and 1:0 (100%).

Solutions of cadmium chloride ($CdCl_2$) and zinc sulphate ($ZnSO_4$) were used as reference samples, in accordance with published guidelines (Environment Canada 2011; OECD 2011) to validate the accuracy of the tests.

Adult sea urchins were collected from a clean intertidal zone at Aguiño (A Coruña, North West Spain). *Isochrysis aff. galbana* (Clon T-ISO) was obtained from the algal collection held in the Department of Microbiology and Parasitology (University of Santiago de Compostela).

The bacterial bioluminescence (*V. fischeri*), microalgal growth (*I. galbana*), and sea urchin larval development (*P. lividus*) tests were conducted following the method described by Carballeira et al. (2012b, d). Bacteria were exposed to diluted effluent samples for 30 min. The 2% diluent was replaced with 3.4% clean seawater (control) to prevent changes in toxicity due to differences in salinity caused by dilution, as we noted that bioluminescence emitted by bacteria in the 2% diluent was half of that emitted in the control seawater, which has the same salinity as the farm discharges.

Regarding potentially confounding factors (salinity, pH, DO, and phosphates), all effluents were generally within the optimal range for the organisms tested (Böttger and McClintock 2001; Saco-Álvarez et al. 2010).

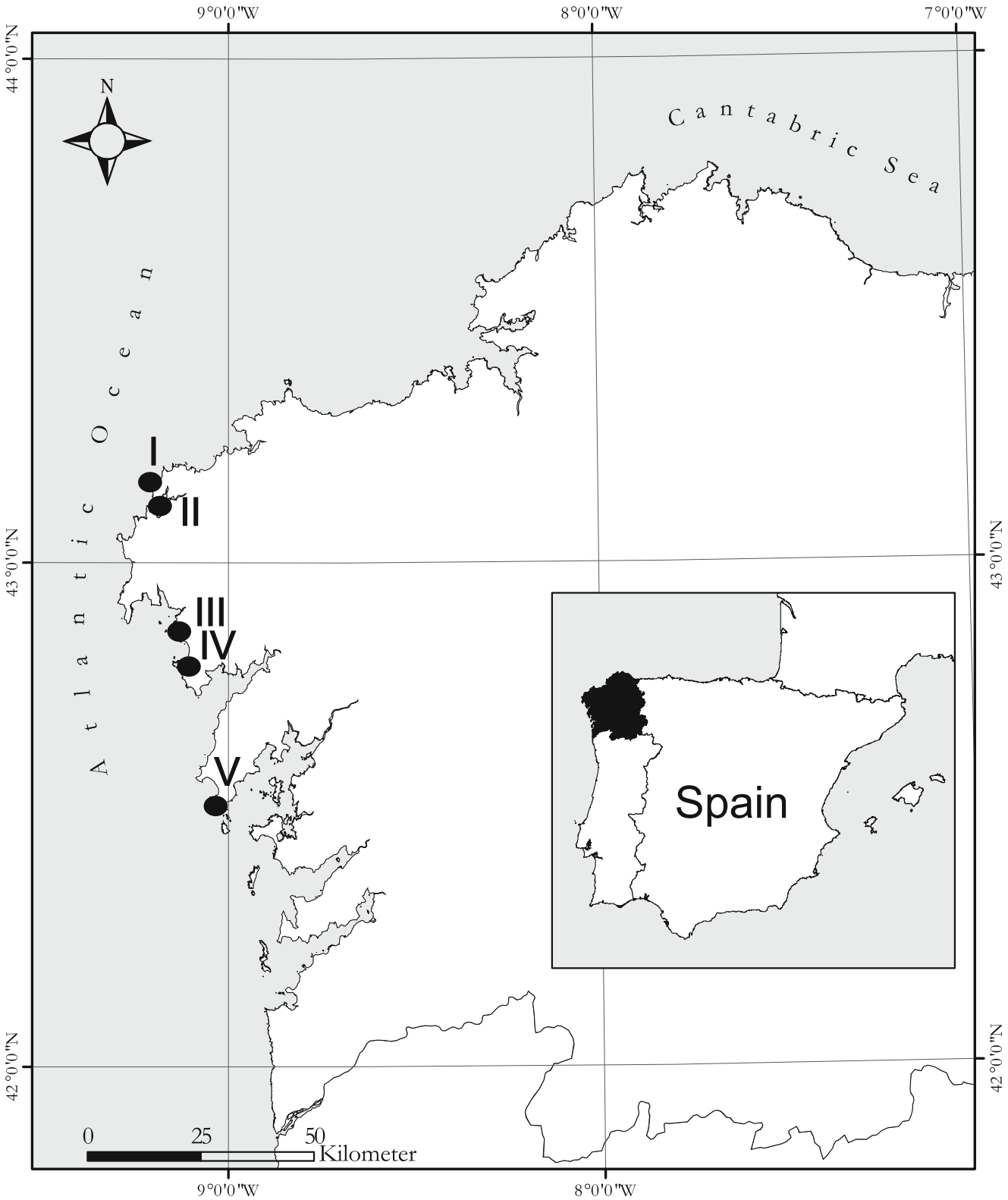

Fig. 1 The locations of the five land-based marine fish farms (I to V) in Galicia (north-west Spain)

Table 1 Averaged physico-chemical characteristics of the effluents ($n = 5$) from land-based fish farms I to V at two different sampling times. The control samples consisted of clean seawater (see text for details)

Month	Sample	Temperature (°C)	Turbidity (ntu)	pH	Conductivity (mS)	Dissolved O_2 (mg L^{-1})
April	Effluent	12.3 ± 0.6	2.7 ± 0.6	7.8 ± 0.0	12.5 ± 0.2	4.0 ± 0.0
	Control	14.0	0.0	7.9	12.5	3.3
July	Effluent	14.8 ± 0.6	1.1 ± 0.7	7.1 ± 0.1	21.3 ± 0.7	n.a.
	Control	17.3	0.0	7.1	22.7	n.a.

Statistical analysis, effective concentrations (EC_x), and the PEEP index

The normality of the data distribution was checked by graphical analysis (qq-plot) and the Shapiro–Wilk normality test. Differences in inhibition of the responses of organisms for the different biodegradation times and farms were identified by the non-parametric Kruskal–Wallis test. The Spearman correlation coefficient was used to determine significant relationships between the responses of organisms and effluent dilutions. Differences were considered statistically significant at $p < 0.05$.

Dose–response curves were created using the dose–response curves (drc) add-on package (Ritz and Streibig 2005). Toxic effects were calculated (when possible) by use of the R software (R Development Core Team 2008) and expressed as effective concentrations (EC_5, EC_{10}, EC_{20}, EC_{50}).

The PEEP index expresses the toxic potential of discharges by combining the results of toxicity bioassays and taking into account the persistence of toxicity, (multi)specificity of toxic impact, and the effluent flow (Costan et al. 1993).

The following formula was used by Costan et al. (1993) to calculate the following index:

$$PEEP = \log_{10}\left[1 + n\left[\frac{\sum_{i=1}^{N} Ti}{N}\right] Q\right]$$

where n = number of samples showing a toxic response, N = total number of samples, T = percentage of resulting toxicity determined by bioassays before and after being biodegraded (expressed as EC_x), and Q = effluent flow ($m^3\ h^{-1}$) (obtained in the present study by written communication with the regional government).

However, this formula underestimates the changes in toxicity resulting from biodegradation of the effluents when applying the logarithm to the difference in toxicity between the time of collection and after degradation for 120 and 240 h. Therefore, in order to obtain more detailed information about the persistence of the toxicity of discharges, we calculated the PEEP as the difference in the value determined independently for the day of the sampling and the value determined after degradation of samples for 120 or 240 h.

Results and discussion

Monitoring intensive aquaculture effluents is a complex task as the discharges are generally of low toxicity and are rapidly dispersed from the coast and, therefore, often only sporadic discharges are detected (Tello et al. 2010). Moreover, the chemical composition of discharges is not usually known because of the secrecy of fish farm producers and because the levels of contaminants do not always reach the detection limits of analytical methods, thus hampering identification of the causes of the changes in the surrounding aquatic environment (Carballeira et al. 2012a, b).

Monitoring demanded by the regional government involves determination of physico-chemical parameters of input water and discharges; however, this is not adequate for assessing the toxicological effects caused by the chemical interaction between contaminants or by the interaction between organic matter and contaminants, and it also does not indicate the bioavailability of contaminants (Mitchell et al. 2002). The physico-chemical parameters of farm effluents were not significantly different (with the exception of turbidity) from those of the input or control water (Tables 1 and 2). Occasional

Table 2 BOD_5 values and NH_4^+ concentrations in effluents (Outflow, $n = 4$) and in the corresponding input water (Inflow, $n = 1$) from land-based fish farms I to V. Values are expressed in milligram per liter

Farm	I		II		III		IV		V	
Parameter	Inflow	Outflow	Inflow	Outflow	Inflow	Outflow	Input	Outflow	Inflow	Outflow
BOD_5	1	4	2	3	2	1	2	4	1	3
NH_4^+	0.089	0.288 ± 0.04	0.001	0.254 ± 0.07	0.064	0.214 ± 0.09	0.066	0.201 ± 0.04	0.006	0.253 ± 0.02

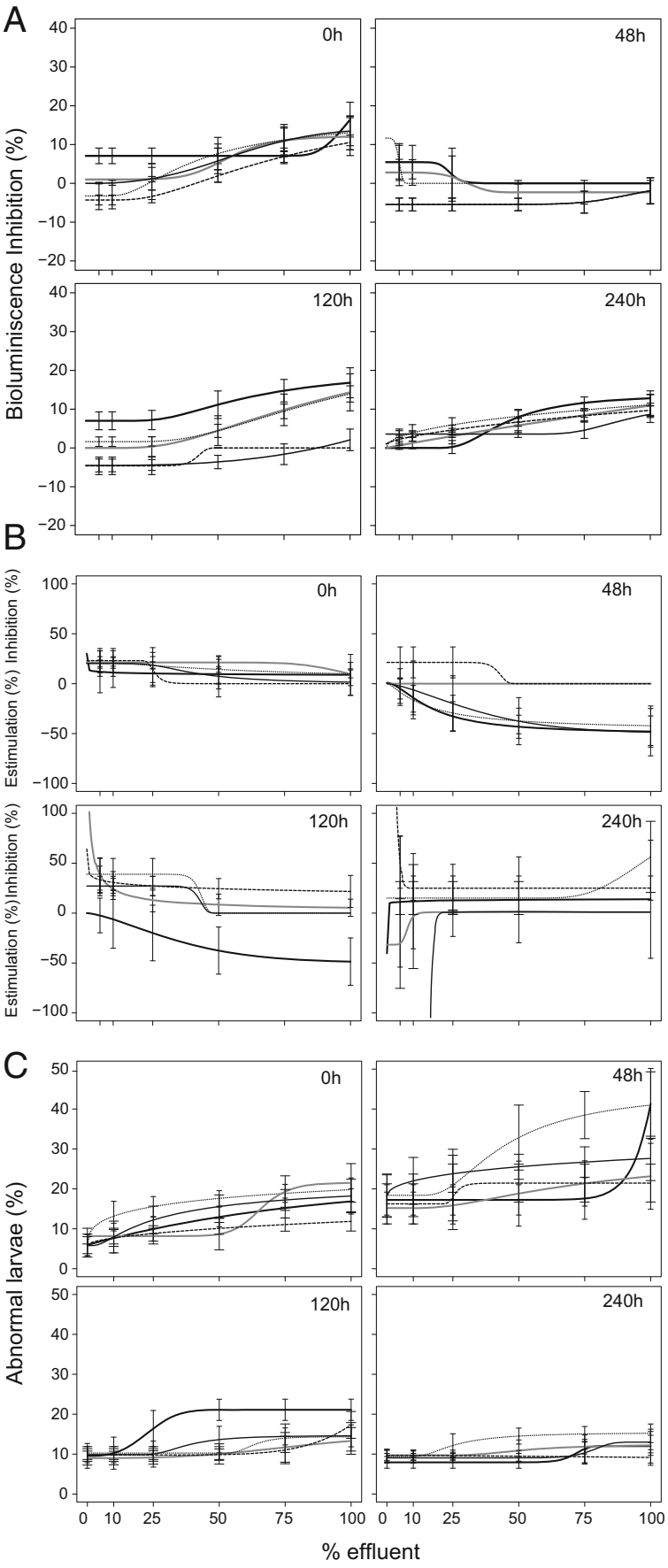

Fig. 2 Dose–response curves for (**a**) the *Vibrio fischeri* bioluminescence bioassay, (**b**) the *Isochrysis aff. galbana* growth test, and (**c**) the *Paracentrotus lividus* larval developmental test for each effluent after being subjected to the biodegradation procedure for 0, 48, 120, and 240 h. Farms are represented by different lines, as follows: I, solid thin line; II, dashed line; III, solid thick line; IV, dotted line, and V, gray line

measures of these parameters do not take into consideration the interannual variability in farm discharges; however, the mean values for a 6-year sampling period also indicated almost no differences throughout the whole period (Carballeira et al. 2012a, b). Although sampling was only occasional, farm effluents were collected during the hours of greatest activity on the farms (between 8 a.m. and 3 p.m.) when facilities are cleaned and fish are fed. In addition, the aim of this study was to determine any changes in toxicity due to biodegradation and not to assess the variability in the toxicity of discharges.

Table 3 Spearman's correlation coefficient relating organism effects to the dilution factor for different biodegradation times (0, 48, 120, and 240 h). Kruskal–Wallis test showing the differences in toxic effects between farms at different biodegradation times (above) and between different biodegradation times on all farms (below). Significant differences are marked in bold type and are indicated by asterisks, as follows: * ($p < 0.05$), ** ($p < 0.005$), and *** ($p < 0.001$)

Farm	Time (h)	Spearman correlation		
		V. fischeri	*I. galbana*	*P. lividus*
I	**0**	**0.511***	**−0.581***	**0.842***
	48	0.075	**0.793*****	**0.372***
	120	**0.762*****	−0.012	**0.79***
	240	**0.908*****	0.28	0.73
II	**0**	**0.674****	−0.266	**0.811***
	48	0.54	0	**0.375***
	120	**0.806*****	**−0.522***	0.364
	240	**0.884*****	0.353	0.041
III	**0**	**0.868*****	**−0.712****	**0.85***
	48	0.159	**−0.618****	**0.806***
	120	**0.774*****	**−0.577***	0.401
	240	**0.835*****	0.214	**0.001***
IV	**0**	**0.856*****	**−0.6***	**0.845***
	48	0.116	**−0.66****	0.551
	120	**0.655****	**−0.628****	**0.638***
	240	0.486	**0.631****	0.651
V	**0**	**0.906*****	**−0.491***	**0.702***
	48	0.386	−0.35	**0.075***
	120	**0.498***	−0.322	0.513
	240	**0.876*****	−0.163	0.282
	Kruskal–Wallis test			
	0	**0.034***	**0.034***	0.112
	48	**0*****	**0.002****	**0.015***
	120	**0*****	**0.033***	**0.01***
	240	0.434	0.053	**0.013***
I		**0.001****	**0*****	**0*****
II		0.051	**0*****	**0*****
III		**0.016***	**0*****	**0*****
IV		**0*****	**0*****	**0*****
V		**0*****	**0.016***	**0*****

The biodegradable organic matter is expressed in terms of biological oxygen demand (BOD_5). The BOD_5 values for effluents reached 4 mg O_2 L^{-1} and were always higher than for input water (between 1 and 2 mg O_2 L^{-1}) (Table 2), except for effluent from farm III whose decomposing activity may have been inhibited by the presence of toxins, probably disinfectants. The BOD_5 values did not surpass the maximum allowed values from administrations and are similar to those obtained in related studies (Sindilariu et al. 2009; Wu et al. 1994).

The bioassays performed have been shown to be useful, sensitive tools for detecting the effects of contaminants in complex water samples, such as those obtained from fish farms (Mitchell et al. 2002). *Vibrio fischeri* is particularly sensitive to antibiotics and disinfectants, whereas *I. galbana* enables the eutrophication potential to be determined, and *P. lividus* larvae are especially sensitive to metals and low pH values (Carballeira et al. 2012c; De Orte et al. 2013). The larvae are highly sensitive to this type of effluent and specific contaminants, as indicated by the appearance of skeletal deformities (Carballeira et al. 2012d). Nevertheless, these bioassays proved to be less sensitive to effluents from the same farms than in previous studies (Carballeira et al. 2012b, d).

The bacterial dose–response curves (Fig. 2a) showed that significant inhibition ($p < 0.05$) of bioluminescence was directly correlated with effluent dilution at 0, 120, and 240 h (Table 3). However, at 48 h, the opposite trend was observed. Similar results were observed with the microalgae (Fig. 2b) as at 48 h there is trophic effect also decreased significantly with increasing dilution (Table 3). No clear trends were observed at the other times. The observed stimulation may occur via a phenomenon called hormesis, as a result of low concentrations of contaminants and the high presence of nutrients, enhancing the physiological activity of biomonitors (Calabrese and Baldwin 1999; Morales-Fernández et al. 2014). These contaminants may later interact with other chemicals or organic matter to form more toxic compounds (Emmanuel et al. 2004).

The percentage of deformed larvae increased significantly with the effluent concentration for all times considered (Table 3 and Fig. 2c). A peak of 40% of deformed larvae occurred at 48 h due to the immaturity of sea urchin eggs. This bioassay was previously reported as the most sensitive to this type of discharge when skeletal deformities of larvae were taken into account (Carballeira et al. 2012d). However, some specific deformities were not observed, which may indicate a change in the chemicals being used in farm management. Statistical analysis has shown differences in toxicity between dilutions, especially when effluents were tested immediately after sampling (0 h) (Table 3), when the toxicity is (presumably) strongest. In addition, no differences between farms were observed in the test performed after 240 h, and differences associated with degradation times were found on almost all farms and in almost all bioassays (Table 3). Together, these results showed changes in toxicity throughout

Table 4 Effective concentrations (ECs) and the estimated errors (in brackets) obtained from dose–response curves for effluents from fish farms I to V generated according to the results of the *Vibrio fischeri*, *Isochrysis galbana*, and *Paracentrotus lividus* bioassays. Values are expressed as effluent dilutions percentage. n.a., not available

Farm	t (h)	*V. fischeri*				*I. galbana*				*P. lividus*			
		EC_5	EC_{10}	EC_{20}	EC_{50}	EC_5	EC_{10}	EC_{20}	EC_{50}	EC_5	EC_{10}	EC_{20}	EC_{50}
I	0	n.a.	91.3 (± 163.2)	104.3 (± 283.3)	n.a.	3400	27.1 (± 47.3)	0.002 (± 0.099)	n.a.	n.a.	25.3 (± 44.4)	190.4 (± 722.8)	n.a.
	48	19.7 (± 41.8)	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	86.2 (± 80)	102.1 (± 82)
	120	n.a.	43.8 (± 19)	262.8 (± 633.3)	n.a.	3.5	n.a.	n.a.	n.a.	n.a.	8.6 (± 10)	34.5 (± 12.1)	n.a.
	240	40.5 (± 3.9)	60.3 (± 12.8)	n.a.	n.a.	6.5	5.8	5.4	4.9	n.a.	71.1 (± 16.8)	n.a.	n.a.
II	0	49.3 (± 8.2)	67.1 (± 27.1)	n.a.	n.a.	109.3 (± 46.9)	99.5 (± 9.7)	77.0 (± 94.1)	n.a.	n.a.	56.2 (± 14.4)	77.6 (± 11)	n.a.
	48	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	60.7 (± 271.3)	n.a.
	120	52.4 (± 61)	75.8 (± 132.9)	143.6 (± 400.8)	n.a.	113.3 (± 400.9)	37.8 (± 131.7)	13.4 (± 46.9)	3.2 (± 11.8)	n.a.	49.1 (± 44.2)	n.a.	n.a.
	240	42.4 (± 277)	90.4 (± 597.1)	196.7 (± 1314.8)	596.8 (± 4064.8)	n.a.	n.a.	n.a.	n.a.	n.a.	36 (± 18.5)	n.a.	n.a.
III	0	38.7 (± 20.6)	64.4 (± 60.9)	n.a.	n.a.	208.0 (± 107.3)	96.3 (± 26.0)	10.5 (± 12.1)	n.a.	n.a.	2 (± 6.3)	106.5 (± 605.1)	n.a.
	48	5.1 (n.a.)	3.6 (n.a.)	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	22.8 (± 10.1)	n.a.
	120	53.1 (± 20.2)	77.8 (± 50.6)	149.5 (± 168.7)	n.a.	45.5 (± 95.2)	44.5 (± 96.3)	43.0 (± 100.1)	n.a.	n.a.	n.a.	n.a.	n.a.
	240	17.2 (± 69.4)	78.5 (± 319.3)	406.1 (± 1690.8)	9714.7 (± 43,532.9)	n.a.	n.a.	73.5	96.0	n.a.	15.5 (± 6.6)	n.a.	n.a.
IV	0	46.5 (± 8.4)	69.5 (± 20.1)	n.a.	n.a.	60.2 (± 54.9)	42.4 (± 23.4)	19.7 (± 24.5)	n.a.	n.a.	16.1 (± 14)	200.3 (± 775.6)	n.a.
	48	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	1.9 (± 12.5)	n.a.
	120	113.8 (± 1106.9)	132.7 (± 1297.6)	162 (± 1595.4)	230.5 (± 2300.7)	44.7 (± 158.4)	43.5 (± 153.2)	41 (± 146.6)	n.a.	n.a.	24.8 (± 22.5)	n.a.	n.a.
	240	77.6 (± 169.8)	113.7 (± 1056.6)	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	73.2 (± 8.2)	n.a.	n.a.
V	0	64.5 (± 38.2)	95.6 (± 79.3)	269.8 (± 394.7)	n.a.	28.6 (± 16.8)	26.5 (± 7.1)	22.4 (± 10.9)	n.a.	n.a.	47.6 (± 364.3)	673.6 (± 5457.6)	n.a.
	48	n.a.	n.a.	n.a.	n.a.	44.3 (± 62.4)	42.7 (± 65.7)	37.2 (± 84.9	n.a.	n.a.	n.a.	27.6 (± 13.9)	n.a.
	120	n.a.	n.a.	n.a.	n.a.	16,502 (± 136,140)	2498 (± 22,182)	155.2 (± 4260)	0.016 (± 1.5)	n.a.	57.2 (± 166.8)	105.2 (± 357.9)	142.7 (± 523.3)
	240	29 (± 74.6)	106 (± 288.5)	433.9 (± 1262.1)	22,738.6 (± 80,710.4)	n.a.	n.a.	n.a.	5 (n.a.)	785.4 (± 5122.6)	n.a.	n.a.	n.a.

Table 5 PEEP index values, calculated by the two different methods, from the EC_{10} values obtained in the different bioassays applied to each fish farm effluent

	Costan et al. (1993)		Proposed modification				
Farm	$P_{0–120\ h}$	$P_{0–240\ h}$	$P_{0\ h}$	$P_{120\ h}$	$P_{240\ h}$	$P_{0\ h}$–$P_{120\ h}$	$P_{0\ h}$–$P_{240\ h}$
I	5.23	5.31	5.31	5.33	6.42	−0.03	−1.12
II	4.91	4.83	4.91	5.06	4.7	−0.15	0.22
III	6.14	6.14	6.22	4.87	5.21	1.35	1.01
IV	5.32	5.24	5.32	5.17	4.5	0.15	0.83
V	4.65	4.55	4.73	3.96	3.38	0.77	1.35

the degradation procedure and the disappearance of toxicity after 10 days.

The dose–response curves only allowed the determination of a few ECs due to the low toxicity of the samples and the high error of models, which may not be correctly adapted to the study conditions (Table 4). The EC values differed depending on the species. Thus, in the case of *V. fischeri*, the toxicity tended to decrease over time, while in the case of *I. galbana*, lower EC values were found at 120 h. By contrast, *P. lividus* showed lower EC values after biodegradation for 48 h, although the general trend in all bioassays was for the toxicity to decrease over time. The toxic effect on *P. lividus* larvae completely disappeared after 240 h (Fig. 2c).

The PEEP indices were calculated with EC_{10} values, according to the formula proposed by Costan et al. (1993) and the method proposed in this study (Table 5). The present method has shown to better reflect the temporal changes in toxicity due to degradation processes. The toxicity of discharges from farm I increased after 240 h, whereas the opposite occurred on farms II, IV, and V. The method proposed by Costan et al. (1993) applies the logarithm to the combination of immediate and delayed (persistent) toxicity, but it overlooks the changes in toxicity. The values obtained with the existing method were very similar to those obtained when no degradation was considered ($P_{0\ h}$), and thus application of the biodegradation procedure would not be necessary in this case.

The low toxicity of farm effluents does not rule out the existence of environmental impacts. The high volumes and types of compounds released may have chronic effects on exposed communities, depending on how the compounds persist in the aquatic environment (Boethling et al. 2009). The most common contaminants in the effluents are ammonia (derived from fish metabolism and decomposition of organic matter), antibiotics (used to treat and prevent diseases), and metals contained in antifouling agents, disinfectants, medicines, and feed (Burridge et al. 2010).

Ammonia is especially harmful to aquatic life at high pH and temperatures, as these conditions lead to the appearance of larger amounts of unionized forms (NH_3); however, ammonia is rapidly oxidized to nitrites and nitrates by bacteria and/or taken up by algae (Hargreaves and Tucker 2004; Crab et al. 2007). Oxidized nitrogenous and phosphate forms were always below the maximum levels required by the national regulations for drinking water (Table 2). However, the levels of ammonia were much higher in the discharges than in the inflowing water, with values close to 0.3 mg L^{-1}. Concentrations of unionized ammonia higher than 0.05 mg L^{-1} can reduce the fertility of fish and increase their susceptibility to disease, and concentrations higher than 2 mg L^{-1} are lethal to fish (Hargreaves and Tucker 2004; Sergeant 2017).

Antibiotics used in aquaculture may persist in sediments (Burridge et al. 2010); however, there is no sediment in the sites where the farms under study are located (Carballeira et al. 2012a), and the half-life of antibiotics in water is much lower, especially when high levels of oxygenation occur, as in areas affected by farm effluents (Deng et al. 2012). Furthermore, the main environmental risk associated with antibiotics is the creation of resistant forms of bacteria, rather than any intrinsic toxicity of the compounds (Wu et al. 1994; Carballeira et al. 2012a).

The persistence of the effects of disinfectants (mainly formaldehyde and chlorinated compounds) in water depends on the levels of ammonia, which may form more toxic compounds called disinfection by-products (DBPs) (e.g., bleach and ammonia may form hydrazine, N_2H_4). Formaldehyde is very toxic to phytoplankton and has a half-life of 36 h in water (De Orte et al. 2013; Lalonde et al. 2015). DBPs and formaldehyde are easily transformed through chemical, biochemical, and photolytic processes into less toxic forms (Emmanuel et al. 2004; Zaidi and Imam 2008), which may explain why toxic effects were mainly observed at the beginning of the assays in the present study.

Metals are very toxic, persistent, and are rapidly accumulated by organisms (Burridge et al. 2010). Copper is used in aquaculture as a molluscicide and algicide, and its toxicity increases with salinity (unionized) and when it is not adsorbed by organic matter (Guardiola et al. 2012). Although Zn is less toxic and persistent than Cu, marine algae are particularly sensitive to its effects (Burridge et al. 2010). The almost total lack of effects on microalgae ruled out the presence of high levels of these metals in the fish farm effluents under study. Moreover, previous accumulation studies did not find significant levels of Cu and Zn in intertidal, sessile organisms in the surroundings of the farms under study (Rey-Asensio et al. 2010).

Study of the long-term effects of fish farm effluents is hindered by the low levels of contaminants in effluents, the secrecy of fish farm companies, and the fact that the aquaculture industry is one of the few that discharge seawater (and most studies of this type concern fresh water). Furthermore, environmental studies on aquaculture activities are mainly based on analysis of sediment, which integrates contaminants, but no methods have been developed for hard-bottom habitats.

The main cause of effluent toxicity seems to be the high concentrations of ammonia, which is rapidly oxidized into less toxic forms. The levels of persistent contaminants have decreased as a result of the implementation of EU regulations that restrict the use of chemical and because fish farms need clean seawater for successful culture of fish. Nonetheless, chronic effects have previously been described in the field, and good practice, the development of multi-trophic aquaculture, and the use of recirculating water systems are recommended in order to minimize the risk of contaminating marine environments.

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References

American Public Health Association (APHA) (2012) Standard methods for the examination of water and wastewater analysis. 22nd edn. American Public Health Association, Washington DC, p 1496

Boethling R, Fenner K, Howard P, Klecka G, Madsen T, Snape JR, Whelan MJ (2009) Environmental persistence of organic pollutants: guidance for development and review of POP risk profiles. Integr Environ Assess Manag 5(4):539–556. https://doi.org/10.1897/IEAM_2008-090.1

Böttger SA, McClintock JB (2001) The effects of chronic inorganic and organic phosphate exposure on development and biochemical composition of gonads in the sea urchin *Lytechinus variegatus* (Echinodermata: Echinoidea). In: Féral, David (eds) Echinoderm research. CRC Press, Netherlands, p 352

Burridge L, Weis JS, Cabello F, Pizarro J, Bostick K (2010) Chemical use in salmon aquaculture: a review of current practices and possible environmental effects. Aquaculture 306(1-4):7–23. https://doi.org/10.1016/j.aquaculture.2010.05.020

Calabrese EJ, Baldwin LA (1999) The marginalization of hormesis. Toxicol Pathol 27(2):187–194. https://doi.org/10.1177/019262339902700206

Carballeira C, Espinosa J, Carballeira A (2011) Linking 15N and histopathological effects in molluscs exposed *in situ* to effluents from land-based marine fish farms. Mar Pollut Bull 62(12):2633–2626. https://doi.org/10.1016/j.marpolbul.2011.09.034

Carballeira C, Ramos-Gómez J, Martín-Díaz M, DelValls T, Carballeira A (2012a) Designing an integrated environmental monitoring plan for land-based marine fish farms located at exposed and hard bottom coastal areas. J Environ Monit 14(5):1305–1316. https://doi.org/10.1039/c2em10839a

Carballeira C, De Orte M, Viana I, Carballeira A (2012b) Implementation of a minimal set of biological tests to assess the ecotoxic effects of effluents from land-based marine fish farms. Ecotoxicol Environ Saf 78:148–161. https://doi.org/10.1016/j.ecoenv.2011.11.022

Carballeira C, De Orte M, Viana I, DelValls T, Carballeira A (2012c) Assessing the toxicity of chemical compounds associated with land-based marine fish farms: the sea urchin embryo bioassay with *Paracentrotus lividus* and *Arbacia lixula*. Arch Environ Contam Toxicol 63(2):249–261. https://doi.org/10.1007/s00244-012-9769-0

Carballeira C, Ramos-Gómez J, Martín-Díaz L, DelValls T (2012d) Identification of specific malformations of sea urchin larvae for toxicity assessment: application to marine pisciculture effluents. Mar Environ Res 77:12–22. https://doi.org/10.1016/j.marenvres.2012.01.001

Costan G, Bermingham N, Blaise C, Férard JF (1993) Potential ecotoxic effects probe (PEEP)—a novel index to assess and compare the toxic potential of industrial effluents. Environ Toxicol Water Qual 8(2):115–140. https://doi.org/10.1002/tox.2530080202

Crab R, Avnimelech Y, Defoirdt T, Bossier P, Vestraete W (2007) Nitrogen removal techniques in aquaculture for a sustainable production. Aquaculture 270(1-4):1–14. https://doi.org/10.1016/j.aquaculture.2007.05.006

De Orte M, Carballeira C, Viana I, Carballeira A (2013) Assessing the toxicity of chemical compounds associated with marine land-based fish farms: the use of mini-scale microalgal toxicity tests. Chem Ecol 29(6):554–563. https://doi.org/10.1080/02757540.2013.790381

Deng Z, Lin Z, Xiaoming Z, Zhifeng Y, Dayong T, Wang D, Daqiang Y (2012) Model of hormesis and its toxicity mechanism based on *quorum sensing*: a case study on the toxicity of sulfonamides to *Photobacterium phosphoreum*. Environ Sci Technol 46(14):7746–7754. https://doi.org/10.1021/es203490f

Emmanuel E, Keck G, Blanchard JM, Vermande P, Perrodin Y (2004) Toxicological effects of disinfections using sodium hypochlorite on aquatic organisms and its contribution to AOX formation in hospital wastewater. Environ Int 30(7):891–900. https://doi.org/10.1016/j.envint.2004.02.004

Environment Canada (2011) Biological test method: fertilization assay using echinoids (sea urchins and sand dollars), method development and applications. Environmental protection series report, Ottawa, pp. 110

FAO (2016) The state of world fisheries and aquaculture 2016. Contributing to food security and nutrition for all. FAO, Rome, p 200

Guardiola FA, Cuesta A, Meseguer J, Esteban MA (2012) Risks of using antifouling biocides in aquaculture. Int J Mol Sci 13(2):1541–1560. https://doi.org/10.3390/ijms13021541

Hach Company (1997) Water analysis handbook. HACH Company, Loveland, p 1309

Hargreaves JA, Tucker CS (2004) Managing ammonia in fish ponds. South Reg Aquac Cent 4603:8

Lalonde BA, Ernst W, Garron C (2015) Formaldehyde concentration in discharge from land based aquaculture facilities in Atlantic Canada. Bull Environ Contam Toxicol 94(4):444–447. https://doi.org/10.1007/s00128-015-1493-9

MAGRAMA (2016). Producción de acuicultura. MAGRAMA. Ministerio de Agricultura, Alimentación y Medio Ambiente. Accessed 1st Aug 2016. Available at: http://www.magrama.gob.es/es/pesca/temas/acuicultura/produccion-de-acuicultura/

Mitchell E, Burgess J, Stuetz R (2002) Developments in ecotoxicity testing. Rev Environ Sci Biotechnol 1(2):169–198. https://doi.org/10.1023/A:1020842718996

Morales-Fernández L, Fernández-Crehuet M, Espigares M, Moreno E, Espigares E (2014) Study of the hormetic effect of disinfectants chlorhexidine, povidone iodine and benzalkonium chloride. Eur J

Clin Microbiol Infect Dis 33(1):103–109. https://doi.org/10.1007/s10096-013-1934-5

OECD (2011) Test no. 201: freshwater alga and cyanobacteria, growth inhibition test. OECD Publishing, Paris, p 25

Pitta P, Apostolaki E, Tsagaraki T, Tsapakis M, Karakassis I (2006) Fish farming effects on chemical and microbial variables of the water column: a spatio-temporal study along the Mediterranean Sea. Hydrobiologia 563(1):99–108. https://doi.org/10.1007/s10750-005-1593-3

R Development Core Team RDC (2008) R: a language and environment for statistical computing. Vienna, Austria R Foundation for Statistical Computing 1:7

Rey-Asensio A, Carballeira C, Viana IG, Carballeira A (2010) Biomonitorización de los efluentes de piscifactorías marinas instaladas en tierra: bioacumulación de microcontaminantes. In: Rey-Méndez M, Lodeiros C, Fernández-Casal J, Guerra A (eds) Foro dos Recursos mariños e da Acuicultura das Rías Galegas XIII. USC, O Grove, pp 201–218

Ritz C, Streibig JC (2005) Bioassay analysis using R. J Stat Softw 12(5):18

Saco-Álvarez L, Durán I, Lorenzo JI, Beiras R (2010) Methodological basis for the optimization of marine sea-urchin embryo test (SET) for the ecological assessment of coastal water quality. Ecotoxicol Environ Saf 73(4):491–499. https://doi.org/10.1016/j.ecoenv.2010.01.018

Sapkota A, Sapkota AR, Kucharski M, Burke J, McKenzie S, Walker P, Lawrence R et al (2008) Aquaculture practices and potential human health risks: current knowledge and future priorities. Environ Int 34(8):1215–1226. https://doi.org/10.1016/j.envint.2008.04.009

Sergeant C (2017) The management of ammonia levels in an aquaculture environment. Water/Wastewater. Accessed on February (2017). Available at: https://www.pollutionsolutions-online.com/article/water-wastewater/17/cancer-research-uk/the-management-of-ammonia-levels-in-an-aquaculture-environment/1557

Sindilariu PD, Reiter R, Wedekind H (2009) Impact of trout aquaculture on water quality and farm effluent treatment options. Aquat Living Res 22(1):93–103. https://doi.org/10.1051/alr/2009009

Tello A, Corner R, Telfer T (2010) How do land-based salmonid farms affect stream ecology? Environ Pollut 158(5):1147–1158. https://doi.org/10.1016/j.envpol.2009.11.029

Wu RSS, Lam KS, MacKay DW, Lau TC, Yam V (1994) Impact of maritime fish farming on water quality and bottom sediment: a case study in the sub-tropical environment. Mar Environ Res 38(2):115–145. https://doi.org/10.1016/0141-1136(94)90004-3

Zaidi BR, Imam SH (2008) Biodegradability. In: Férard J, Blaise C (eds) Encyclopedia of aquatic ecotoxicology. Springer, Netherlands, pp 357–366