Ecotoxicology and Environmental Safety 78 (2012) 148–161

Contents lists available at SciVerse ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Implementation of a minimal set of biological tests to assess the ecotoxic effects of effluents from land-based marine fish farms

C. Carballeira [a,*], M.R. De Orte [a], I.G. Viana [b], A. Carballeira [c]

[a] Departamento de Química Física, Cátedra UNESCO/UNITWIN/WICOP, Facultad de Ciencias del Mar y Ambientales, Polígono Río San Pedro s/n, 11510 Puerto Real, Cádiz, Spain
[b] Instituto Español de Oceanografía, Centro Costero de A Coruña, 130, 15080, A Coruña, Spain
[c] Departamento de Ecología, Facultad de Biología, Universidad de Santiago de Compostela, 15782, Santiago de Compostela, A Coruña, Spain

ARTICLE INFO

Article history:
Received 2 September 2011
Received in revised form
3 November 2011
Accepted 16 November 2011
Available online 2 December 2011

Keywords:
Pisciculture discharges
Pond
Biomonitoring
Toxicity test
Microtox®
Microalgal growth
Sea urchin larval development

ABSTRACT

Environmental monitoring plans (EMP) that include chemical analysis of water, a battery of bioassays and the study of local hydrodynamic conditions are required for land-based marine aquaculture. In this study, the following standardized toxicity tests were performed to assess the toxicity of effluents from eight land-base marine fish farms (LBMFFs) located on the northwest coast of Spain: bacterial bioluminescence (with *Vibrio fischeri* at 15 and 30 min), microalgal growth (with *Phaeodactyllum tricornutum* and *Isochrysis galbana*) and sea urchin larval development (with *Paracentrotus lividus* and *Arbacia lixula*). These bioassays were evaluated for inclusion in routine fish farm monitoring. Effective concentrations (EC_5, EC_{10}, EC_{20}, EC_{50}) for each bioassay were calculated from dose–response curves, obtained by fitting the bioassay results to the best parametric model. Moreover, a graphical method of integrating the results from the battery of bioassays and classifying the toxicity was proposed, and the potential ecotoxic effects probe (PEEP) index was calculated. The bacterial bioluminiscence test at 30 min, growth of *I. galbana* and larval development of *A. lixula* were found to be the most sensitive and useful tests. Graphical integration of these test results enabled definition of the ecotoxicological profiles of the different farms. The PEEP index, considering EC_{20}, efficiently reflected the toxic loading potential of LBMFF effluents. In conclusion, a battery of bioassays with species from different low trophic levels is recommended as a rapid and cost-effective methodology for assessing LBMFF discharges. The graphical integration method and the PEEP index are proposed for consideration in EMPs for such farms.

1. Introduction

Marine land-based aquaculture has become more intensive over the last 15 years, mainly as a result of the introduction of new technologies, expansion of suitable sites, improvements in feed technology, improved understanding of the biology of the species farmed, increased water quality within farming systems and the increased demand for fish products (Read and Fernandes, 2003). Although the output from fisheries remained constant, flatfish aquaculture increased significantly from 26,300 t in 2000 to 148,800 t in 2008, with China and Spain being the leading producers (FAO, 2010). In Spain, the main species involved is the turbot (*Psetta maxima*), which is produced in land-based facilities.

Fish production can generate considerable amounts of effluents, such as waste food, feces, medications and pesticides, which may have (eco)toxic and trophic effects (eutrophization), and consequently may alter the structure and development of the receiving ecosystems. Discharges from aquaculture to the aquatic environment may be categorized as continuous discharge, periodic discharge and pulses of veterinary medicines and/or their mixtures (Tello et al., 2010). Discharges include a wide range of chemicals involved in feed composition and metabolization, liming materials, algicides, disinfectants, antibiotics, hormones, osmoregulators and probiotics. Most studies of effluents from land-based fish farms have focused on output nutrients, biochemical oxygen demand and suspended solids, but few studies have considered chemicals and pathogens (Tello et al., 2010).

If the understanding of the ecotoxicological effects does not keep pace with the emergence of new aquaculture products, waste water discharges from fish farms may become a problem. A serious environmental impact may occur when untreated effluents are released into the sea. Regulations, methodological guidelines and protocols, and monitoring plans have been developed for marine fish farms (Fernandes et al., 2000; GESAMP, 1996; Hansen et al., 2001; Roque D'Orbcastel et al., 2004; SEPA, 1999; Stigebrandt et al., 2004), although these all refer to the environmental impact caused by marine fish farms installed in

* Corresponding author. Fax: +34 956016040.
E-mail addresses: carlos.carballeira@uca.es (C. Carballeira), manoela.romano@uca.es (M.R. De Orte), inesgviana@gmail.com (I.G. Viana), alejo.carballeira@usc.es (A. Carballeira).

0147-6513/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.ecoenv.2011.11.022

cages. Protocols or environmental monitoring plans (EMPs) for land-based marine fish farms (LBMFFs) are practically non-existent, and there is a critical need to improve the information on inland fishery resources and on the people that use and depend on them (FAO, 2010). Since monitoring of the impacts of cage fish culture mainly focuses on benthic communities, which are largely affected by the type of site where the cages are located and the type of management carried out, other options for the design of EMPs for LBMFFs must be considered.

To date, the organization responsible for the environmental surveillance of LBMFFs in Galicia (northwest Spain) has only considered conventional physicochemical parameters in monitoring the effluents. The information obtained from such analyses is insufficient, because emerging pollutants are not taken into account, and the data do not indicate the potential effects on ecological processes in the water column (Sarà, 2007) because they do not reflect the bioavailability of contaminants (Hernando et al., 2007) and pollutant interactions (e.g. synergistic effects) (Richardson et al., 2007). Furthermore, chemical analysis of trace elements is complex and entails high costs. Evaluation of the relationship between the information obtained by monitoring and the cost of obtaining such information (Borja, 2002) within the *experimental control* versus *environmental realism* concept are necessary when designing a practical EMP. The information compiled should describe the ecological processes and not merely describe local situations (Underwood, 1997).

In this context, the use of laboratory bioassays to evaluate the potential toxicity of fish farm effluents, in combination with study of the hydrodynamic conditions in the area, may provide useful ecotoxicological data, and may also enable estimation of the capacity of the environment to assimilate the impact. Field testing and field monitoring provide more realistic ecological evidence, but cannot always be applied, because of their complexity and high cost (DelValls, 2007). Laboratory bioassays are monitoring tools that try to estimate the likelihood that contaminants within an environmental matrix will cause effects at different levels of organization and, ultimately, harm the surrounding ecosystem. Laboratory bioassays are relatively simple to carry out and therefore should be considered in EMPs with the same frequency as conventional chemical analyses.

Many regulatory agencies assess the environmental toxicity of discharges by use of a battery of test organisms that may include aquatic invertebrates, fish, bacteria, microalgae and higher plants (OECD, 1998; USEPA, 2000). A meaningful battery of bioassays with high sensitivity to pollutants for the evaluation of effluents and superficial waters should ideally include test species belonging to different trophic levels: producers (algae and higher plants), consumers (crustaceans and rotifers) and decomposers (Mankiewicz-Boczek et al., 2008) so that the toxicological profile will be better understood. The selection of test species is determined by their relevance, prevalence, accessibility, ease of maintenance and culture, cost-effectiveness and by how easy they are to observe and quantify (Jiangning et al., 2004).

The bioluminescence inhibition test that uses the bacteria *Vibrio fischeri* has been widely applied in water analysis and to evaluate the toxicity of biocides, which are the same or similar to those used in fish farming (Backhaus and Grimme, 1999; Coelho et al., 2011; Hernando et al., 2007; Isidori et al., 2005; Lalumera et al., 2004; Muñoz et al., 2010; Park and Choi, 2008). Microalgae have been recommended as test organisms because of their ecological relevance and sensitivity (Pavlic et al., 2006; Satoh et al., 2005). Toxicity tests conducted with embryos of invertebrates are widely used because of their sensitivity to chemicals of different nature, ease of handling, low cost and applicability in both laboratory and field conditions (Beiras et al., 2001).

According to this, the first aim of this study was to apply a set of marine bioassays to evaluate the impact of LBMFFs on marine life. This set of tests comprised the bioluminescence inhibition test with the bacterium *V. fischeri*, the embryo development test with the sea urchin species *Paracentrotus lividus* and *Albacia lixula*, and the microalga growth test with the species *Phaeodactyllum tricornutum* and *Isochrysis galbana*. A minimal battery of bioassay was thus proposed for assessment of the effects of discharges from LBMFFs on decomposers (bacteria), primary producers (microalgae) and primary consumers (sea urchins). Because of the lack of knowledge of the impact generated by this type of aquaculture, the use of two different species of microalgae and sea urchins and different exposure times with *V. fischeri* were tested to determine the most suitable assay in accordance with the physicochemical conditions of each farm.

The second objective was to propose, through prior evaluation, a toxicity classification system based on the aforementioned battery of bioassays for routine integral biological monitoring of LBMFFs.

2. Material and methods

2.1. Effluent characteristics

This study focused on the effluents generated by eight LBMFFs (I, II, III, IV, V, VI, VII and VIII) located in Galicia (northwest Spain; Fig. 1). These farms grow *Psetta maxima* and *Solea solea* and they account for approximately the 50% of the production of these species in Spain (APROMAR, 2011). Significant differences in the level of production and the volume of residual water generated can be found amongst each other (Table 1).

The Galician agency responsible for the environmental monitoring of the LBMFFs provided the physicochemical characterization of the effluents from the fish farms (Table 1). The same characteristics were measured in the input (I) and in the output (O) water from 18 LBMFFs situated in the same region during the period 2002–2008, and the average values obtained for the samples were similar to those obtained in the effluents tested (Table 2).

The presence of specific contaminants was evaluated by means of bioaccumulation measurements in several native macroalgae (*Fucus* sp. and *Codium tomentosum*) and in transplanted specimens of the macroalga *Saccharina saccharina* (Rey-Asensio et al., 2010). Metals are major constituents of disinfectants and anti-fouling products and are present in the diet of cultivated fish (Dean et al., 2007). There were no significant differences ($p < 0.05$) in the bioaccumulation of the most important metals (Cd, Cr, Cu, Pb and Zn) in either of the species within a gradient of sampling sites affected by LBMFF discharges (Rey-Asensio et al., 2010). All metal concentrations were below the mean background concentration determined for meso-polyhaline waters (Tueros et al., 2008). The concentrations of the antibiotics sulfadiazine, flumequine, oxolinic acid, oxytetracycline and amoxicillin were found to be below the detection limit of chemical analysis in all species, but significant differences ($p < 0.05$) in their bioconcentration were observed in *S. saccharina* collected in the surroundings of the LBMFFs. Some pesticides such as prometryn, prometon and chlorothalonil were also detected in the transplanted algal specimens (Rey-Asensio et al., 2010).

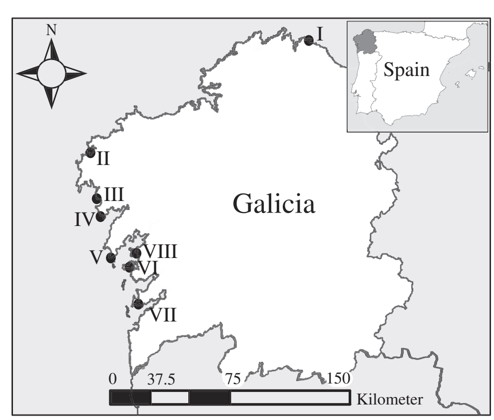

Fig. 1. Location of the eight land-based marine fish farms (designated from I to VIII) on a map of Galicia (northwest Spain).