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# Ecological risk assessment of the antibiotic enrofloxacin applied to *Pangasius* catfish farms in the Mekong Delta, Vietnam

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## HIGHLIGHTS

- We assessed the risks of enrofloxacin (ENR) and its main metabolite ciprofloxacin (CIP).
- Maximum ENR and CIP concentrations in aquaculture effluents were 0.68 and 0.25 μg $L^{-1}$.
- ENR and CIP accumulated in sediments down-stream the effluent discharge point.
- Tropical freshwater organisms showed high tolerance to ENR and CIP exposure.
- Only microbial communities are expected to be affected by ENR and CIP residues.

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## ABSTRACT

Antibiotics applied in aquaculture production may be released into the environment and contribute to the deterioration of surrounding aquatic ecosystems. In the present study, we assessed the ecological risks posed by the use of the antibiotic enrofloxacin (ENR), and its main metabolite ciprofloxacin (CIP), in a *Pangasius* catfish farm in the Mekong Delta region, Vietnam. Water and sediment samples were collected in a stream receiving effluents from a *Pangasius* catfish farm that had applied ENR. The toxicity of ENR and CIP was assessed on three tropical aquatic species: the green-algae *Chlorella* sp. (72 h – growth inhibition test), the micro-invertebrate *Moina macrocopa* (48 h – immobilization test), and the Nile tilapia (*Oreochromis niloticus*). The toxic effects on *O. niloticus* were evaluated by measuring the cholinesterase (ChE) and catalase (CAT) activities in the fish brain and muscles, respectively, and by considering feed exposure and water exposure separately. Ecological risks were assessed by comparing maximum exposure concentrations with predicted no effect concentrations for cyanobacteria, green algae, invertebrates and fish derived with available toxicity data. The results of this study showed that maximum antibiotic concentrations in *Pangasius* catfish farm effluents were 0.68 μg $L^{-1}$ for ENR and 0.25 μg $L^{-1}$ for CIP (dissolved water concentrations). Antibiotics accumulated in sediments down-stream the effluent discharge point at concentrations up to 2590 μg $kg^{-1}$ d.w. and 592 μg $kg^{-1}$ d.w. for ENR and CIP, respectively. The calculated EC50 values for ENR and CIP were 111 000 and 23 000 μg $L^{-1}$ for *Chlorella* sp., and 69 000 and 71 000 μg $L^{-1}$ for *M. macrocopa*, respectively. Significant effects on the ChE and CAT enzymatic activities of *O. niloticus* were observed at 5 g $kg^{-1}$ feed and 400–50 000 μg $L^{-1}$, for both antibiotics. The results of the ecological risk assessment performed in this study indicated only minor risks for cyanobacteria communities, suggesting that residual concentrations of ENR and CIP after medication are not likely to result in severe toxic effects on exposed aquatic ecosystems. However, more studies should be performed by considering other antibiotic treatments used in *Pangasius* catfish production and the potential ecotoxicological effects of relevant antibiotic mixtures on sediment communities.



## 1. Introduction

Vietnam is the third largest aquaculture producing country in the world (FAO, 2012). The Vietnamese aquaculture production sector has sharply increased mainly due to the development and

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expansion of *Pangasius* catfish (*Pangasius hypophthalmus*) production in the Mekong River Delta (Phan et al., 2009), which has increased ten-fold in the last decade, reaching an annual production of 1.14 million tonnes in 2012 (FAO, 2012). The success of this aquaculture species is explained by its ability to be reproduced in captivity, its fast growth, its capability to tolerate low water oxygen concentrations, the development of improved culture and feeding techniques, and the expansion of the export markets (Phuong and Oanh, 2010). *Pangasius* catfish are produced in earthen ponds with relatively high water depth (3.5–4.5 m), which are stocked with exceptionally high fish densities (about 50 fish $m^{-2}$) and rely on heavy water exchange regimes with the surrounding aquatic ecosystems – once or twice a day, from 30% to 100% replenishment (Phan et al., 2009). The intensification of *Pangasius* production practices has been accompanied by the outbreak of several bacterial and parasitic infestations, which has led to the introduction of a wide array of veterinary medicines for their prevention and treatment (Phan et al., 2009; Rico et al., 2013). Eventually, residual concentrations of veterinary medicines used in *Pangasius* production can be released into the environment by untreated effluent and sludge discharges, and have raised concerns about their potential toxic effects on aquatic ecosystems surrounding the aquaculture farms (Thuy et al., 2011; Rico et al., 2012). Several studies have warned about the spread and the high levels of antibiotic pollution in the aquatic ecosystems of the Mekong Delta due to the input of aquaculture, livestock and urban effluents (Managaki et al., 2007; Shimizu et al., 2013). However, the contribution of the *Pangasius* catfish farms to this pollution problem and their potential ecological consequences have not been investigated so far. A recent modelling study identified the *Pangasius* farming areas of the Mekong Delta as potential hot-spots for environmental pollution due to their widespread use of veterinary medicines and their intensive discharge of untreated effluents, and stressed the need to monitor and further assess the ecological effects of selected aquaculture antibiotics on streams impacted by *Pangasius* catfish effluents (Rico and Van den Brink, 2014).

The aim of this study was to get a better understanding on the environmental fate and ecological risks posed by the use of antibiotics in *Pangasius* catfish production. For this, we evaluated the discharge of the fluoroquinolone antibiotic enrofloxacin (ENR) and its main metabolite ciprofloxacin (CIP) on freshwater ecosystems surrounding *Pangasius* catfish farms and assessed their potential toxicological effects on tropical freshwater ecosystems. ENR was chosen as model compound because of its widespread use in *Pangasius* production as well as in other important aquaculture species produced in Asia (Rico et al., 2013). In this study, the concentrations of ENR and CIP were monitored in environmental samples collected in a tropical stream receiving effluents from a *Pangasius* farm during and after ENR treatment. Moreover, the toxicological effects of these antibiotics were assessed on tropical aquatic organisms representing three different trophic levels: the algae *Chlorella* sp., the invertebrate *Moina macrocopa*, and the fish *Oreochromis niloticus*. Finally, the ecological risks posed by the use of ENR in *Pangasius* catfish farms were assessed by comparing the concentrations measured in the field with the predicted no effect concentrations derived with toxicity data for aquatic organisms.

## 2. Material and methods

### 2.1. Antibiotic exposure assessment

#### 2.1.1. Sample collection

This study was conducted in February of 2012 in a *Pangasius* catfish pond of 0.26 ha, with an average water depth of 3.3 m. At the moment of the antibiotic treatment, the pond contained approximately 10 tons of fish, with an individual size distribution of 103 ± 9.1 g (mean ± SD). ENR was administered daily mixed with feed at a dose of approximately 10 mg $kg^{-1}$ of fish body weight for a period of 5 d, according to the dosages typically reported by *Pangasius* farmers (Rico et al., 2013). ENR was diluted in water and sprayed over the commercial pelleted feed (28% protein content), and mixed manually. About 22% of the pond's water was daily replaced by tidal flushing. The draining water was discharged by two pipes into a natural stream of approximately 3.7 m width, 0.7 m depth, and with an average water flow of 0.15 m $s^{-1}$. Water and sediment samples were collected in the two water discharge points of the pond (DP1 and 2) after the first application (day 0), after the third application (day 3) and on days 1, 3, 7, 14 and 21 after the last antibiotic application. Extra water and sediment samples were collected one day after the last application in 5 sampling points (separated by 25 m) located in a longitudinal transect of the stream receiving the farm effluents (S1–5) (Fig. 1). Water samples were taken at 10 cm depth in previously rinsed plastic bottles. Depth integrated (5 cm) sediment samples were taken with a core sampler and immediately introduced into zip lock plastic bags. All samples were kept in a cooler during transportation and stored until further analysis (water samples: 4 °C; sediment samples: −20 °C).

#### 2.1.2. Determination of antibiotic concentrations

The dissolved antibiotic concentrations ($C_{diss}$) in the water samples were determined by LC-MS/MS according to the method described in Rico et al. (2014b). The analytical method recoveries were 112 ± 13% and 91 ± 9.6% for ENR and CIP, respectively (mean ± SD; $n = 4$), and the limit of detection (LOD) and quantification (LOQ) were 0.01 and 0.02 μg $L^{-1}$ for ENR, and 0.01 and 0.04 μg $L^{-1}$ for CIP, respectively. The total antibiotic concentration in the water samples ($C_{total}$) was calculated according to the method described in the Supplementary information (SI).

The antibiotic elution and analysis from the sediment samples was performed according to the method described by Rico et al. (2014b). The recoveries of the analytical method at a sediment concentration of 50 μg $kg^{-1}$ d.w. were 99 ± 6% and 84 ± 15% for ENR and CIP, respectively (mean ± SD; $n = 3$), and the LOD and LOQ were 0.3 and 1.1 μg $kg^{-1}$ d.w. for ENR, and 0.7 and 2.3 μg $kg^{-1}$ d.w. for CIP. In order to assess the antibiotic exposure for benthic aquatic organisms, the pore water concentrations ($C_{pore\ water}$) equivalent to the measured sediment concentrations were determined according to the method described in the SI.

### 2.2. Toxicity tests

#### 2.2.1. Toxicity tests with Chlorella sp.

Toxicity tests with ENR and CIP on *Chlorella* sp. were conducted according to OECD (2006). The experiment was performed in triplicate ($n = 3$) with algae cultures ($0.6 \times 10^6$ cells $mL^{-1}$) exposed for 72 h in 250 mL Erlenmeyer flasks to a series of six antibiotic concentrations. The culture media (100 mL) consisted of sterilized tap water, 200 μL of concentrated growth medium prepared according to Walne (1970), and 3 drops of vitamins. Algae were grown in a temperature controlled room (26 ± 1 °C) with constant illumination intensity (provided by neon light). The temperature and pH of the culture media were monitored 1 h, 36 h, and 72 h after the start of the experiment. One mL of the culture media (one per treatment level) was taken for antibiotic analysis right after spiking and at the end of the experimental period. Algae were sampled 1 h, 36 h and 72 h after the start of the experiment from each test unit and were fixated with formaldehyde (4%; v/v). The number of algae cells per mL was counted by using a counting chamber and a powerful microscope (400×). Finally, the average

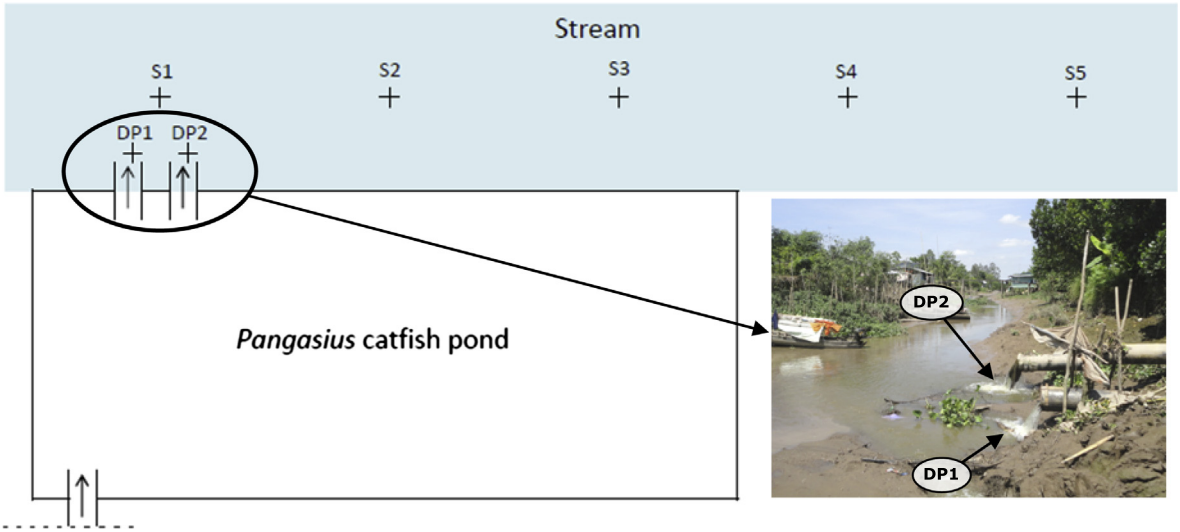


**Fig. 1.** Scheme of the *Pangasius* catfish pond and the water stream receiving the pond effluent, with the selected water and sediment sampling points (crosses). The effluent discharge points are indicated as DP1 and DP2, and the sampling points located in the water stream as S1–5.

specific growth rate and the percentage of growth inhibition were calculated as a function of time for each treatment level (including the controls) according to OECD (2006).

*2.2.2. Toxicity tests with M. macrocopa*

Toxicity tests with ENR and CIP on *M. macrocopa* were conducted according to the OECD 202 standard procedure (OECD, 2004) adapted to the higher temperatures and light regime occurring in tropical ecosystems. *M. macrocopa* neonates (<24 h old) were exposed to a series of six antibiotic concentrations for 48 h with five replicates per treatment level. The tests were performed in 5 mL glass cuvettes containing 3 mL of exposure media, prepared by diluting the stock solutions of antibiotics on filtrated pond water, and with three *M. macrocopa* individuals per test unit. All cuvettes were placed on a platter in the laboratory (temperature: 31 ± 1 °C; 12–12 h light/dark regime). Four extra cuvettes per treatment level were installed in order to collect samples for antibiotic analysis and water quality measurements. One mL water samples were taken for antibiotic analysis (one per treatment level) after spiking and at 24 h and 48 h after the start of the experiment. On the same sampling times, the temperature and pH of the exposure media were monitored and the number of immobilized individuals in each cuvette was recorded. The *M. macrocopa* individuals were considered to be immobile when no movement was observed after 15 s of gentle water agitation.

*2.2.3. Toxicity tests with O. niloticus*

Juvenile *O. niloticus* of 11 ± 2 g (mean ± SD) were obtained from a hatchery and reared in a big composite tank with aeration for one month in order to acclimatize to the food (commercial pelleted feed: 30% protein, 5% fat) and the water used in the experiments. The effects of ENR and CIP on the muscle catalase (CAT) and brain cholinesterase (ChE) activities of *O. niloticus* were studied by performing separate toxicity tests with two different modes of antibiotic administration: antibiotic added mixed with feed (oral administration) and added directly to water (bath treatment). The oral administration experiments were performed in triplicate for a series of antibiotic dosages of 0, 1, 2.5, 5, 10 g ENR $kg^{-1}$ of feed (i.e., representing 0, 30, 75, 150, 300 mg ENR $kg^{-1}$ of fish body weight), and 0, 0.50, 1.12, 2.5, 5.0 g CIP $kg^{-1}$ of feed (i.e., representing 0, 15, 33.6, 75, 150 mg CIP $kg^{-1}$ of fish body weight). Prior to the start of these experiments, small bags of antibiotic-treated and antibiotic-free feed were prepared. For this, stock solutions of ENR and CIP were sprayed over the food pellets and further mixed with a spoon in order to reach the desired antibiotic concentrations. Subsequently, all the feed was placed into a freezer (−20 °C) to avoid antibiotic degradation until the moment of administration. The bath treatment experiments were also performed in triplicate. During the exposure period, stock solutions of ENR and CIP were daily prepared and added to the fish culture media in order to generate the following water concentrations: 0; 100; 800; 10 000; 100 000 μg ENR $L^{-1}$, and 0; 50; 400; 5000; 50 000 μg CIP $L^{-1}$. All experiments lasted for 14 d, with 5 d of antibiotic treatment period and 9 d of post-exposure period (i.e., recovery period). During the exposure and recovery periods, 20 L of test media were daily replaced in order to prevent excessive water quality deterioration. Food was administered two times per day (at 8 am and 5 pm) at a feeding rate of 3% and 5% of fish body weight during the treatment and recovery period, respectively. On day 1, 3, 5, 7, 10 and 14 after the start of the experiment, temperature and pH were monitored in each tank and water samples (1 mL) were taken for antibiotic analysis (one sample per treatment level). On day 3, 5 and 14, after the start of the experiment, three fish per tank were killed by a cold shock in ice and directly dissected to extract their brains and part of their muscles. The samples were stored at −80 °C until further analysis. The methods used for the biomarker activity analysis are described in the SI.

*2.2.4. Chemical analysis*

For information on the method used for the analysis of the antibiotic concentrations in the exposure media of the toxicity experiments see the SI.

*2.3. Data analysis*

The concentrations resulting in 10% and 50% (EC10 and EC50) inhibition of algae growth (for *Chlorella* sp.) and immobilization (for *M. macrocopa*) at the end of the experimental period and their 95% confidence intervals (CIs) were calculated by probit analysis using linear maximum likelihood regression with the ToxRat Professional Version 2.01 software (ToxRat, 2003). All calculations were performed using the measured antibiotic concentrations.

The differences between the enzymatic activities measured in the *O. niloticus* controls and those measured for the different

antibiotic treatment levels were assessed for each sampling date using a one-way analysis of variance (ANOVA) test followed by a Dunnett's post hoc test. In addition, the Kolmogorov–Smirnov test and the Levene's test were performed in order to verify the normality and the variance homogeneity assumptions of the tested data. For the datasets for which these two criteria were not met, the ANOVA and Dunnett's test were substituted by the Kruskal–Wallis test followed by a Dunn's multiple comparison test. These statistical tests were performed using the SPSS statistical package (ver. 19.0, SPSS Company, Chicago, IL, USA). Differences between controls and antibiotic treatments were considered to be statistically significant when $p \leqslant 0.05$.

### 2.4. Ecological risk assessment

The toxicological risks for cyanobacteria, green algae, invertebrates and fish in the aquatic ecosystems surrounding *Pangasius* catfish farms were assessed by following a risk quotient (RQ) approach. RQs were calculated by dividing the highest ENR and CIP measured exposure concentrations by the predicted no effect concentrations (PNECs) for each taxonomic group. The aquatic exposure RQs for algae and cyanobacteria were calculated based on the $C_{diss}$, as they will be more exposed to the freely dissolved antibiotics in the water. For invertebrates and fish, the aquatic exposure RQs were conservatively calculated based on the $C_{total}$, given their capacity to filter and/or feed on suspended organic particles. The sediment exposure RQs were calculated based on the $C_{pore\ water}$. The PNECs were derived by using the toxicity values calculated in this study as well as other toxicity values collected from the literature. PNECs were calculated by dividing the lowest acute EC50 value available for each taxonomic group by an assessment factor (AF) according to VICH (2004).

## 3. Results

### 3.1. Aquatic exposure assessment

Measured antibiotic concentrations in the water samples (dissolved fraction) collected in the pond effluents (DP 1 and 2) ranged between 0.05 and 0.68 μg $L^{-1}$ for ENR, and between <LOD and 0.25 μg $L^{-1}$ for CIP (Table S1). According to our calculations, the total antibiotic concentrations in the pond effluent ranged between 0.24 and 3.15 μg $L^{-1}$ for ENR, and between <LOD and 0.39 μg $L^{-1}$ for CIP. Measured sediment concentrations for ENR and CIP in the effluent discharge points were found to be insignificant (<LOD).

Measured ENR and CIP concentrations in the water samples collected down-stream the effluent discharge point (S1–5) fell below the detection limit. Sediment concentrations showed a gradual increase in the samples monitored down-stream the effluent discharge point, with maximum concentrations being 2590 μg $kg^{-1}$ d.w. for ENR, and 592 μg $kg^{-1}$ d.w. for CIP, in S5 (Fig. 2). The corresponding maximum pore water concentrations were 0.33 μg $L^{-1}$ for ENR and 0.45 μg $L^{-1}$ for CIP.

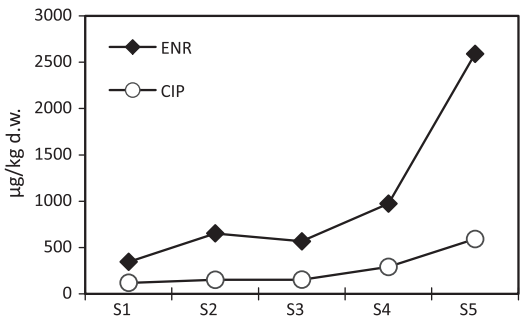


**Fig. 2.** Measured enrofloxacin (ENR) and ciprofloxacin (CIP) concentrations in the sediment samples collected down-stream the effluent discharge point (S1–5).

### 3.2. Toxicity tests

#### 3.2.1. Toxicity tests with Chlorella sp.

In both experiments conducted with *Chlorella* sp., the cultured algae population grew exponentially in the control treatment, and the growth was totally inhibited by the highest antibiotic concentration (Fig. S1). The measured antibiotics concentrations in the test media showed an average dissipation of 22% for ENR and 29% for CIP at the end of the experimental period. The results of the toxicity experiments showed that *Chlorella* sp. is more sensitive to CIP (EC50-72 h = 23 400 μg $L^{-1}$ or 70.6 $\times$ $10^{-6}$ mol $L^{-1}$) than to ENR (EC50-72 h = 111 000 μg $L^{-1}$ or 309 $\times$ $10^{-6}$ mol $L^{-1}$) (Table 1).

#### 3.2.2. Toxicity tests with M. macrocopa

ENR and CIP had similar toxicity to *M. macrocopa* (ENR: EC50-48 h = 69 100 μg $L^{-1}$ or 192 $\times$ $10^{-6}$ mol $L^{-1}$, and CIP: EC50-48 h = 71 200 μg $L^{-1}$ or 214 $\times$ $10^{-6}$ mol $L^{-1}$) (Table 1). In these tests, CIP showed a fast dissipation in the *M. macrocopa* culture media (average 48 h dissipation: 42%), whereas ENR was found to be rather stable (average 48 h dissipation: 2%).

#### 3.2.3. Toxicity tests with O. niloticus

For all CIP tested doses in the oral administration test, CAT activity was not significantly different from the controls during the entire experimental period ($p > 0.05$). A clear trend was observed towards a decrease in CAT activity on the last day of the exposure period (day 5) in the tests performed with ENR, showing a significant effect for the lowest and the highest tested concentrations (Fig. 3A and B). A significant increase of the ChE activity was observed on days 3 and 5 for the fish that were exposed to 5 and 10 g ENR $kg^{-1}$ of feed and 5 g CIP $kg^{-1}$ of feed, however, the ChE activity returned to basal enzymatic levels on day 14 (Fig. 3C and D). In general, the temperature and pH values measured in these experiments remained rather constant during the experimental period (ENR: $T = 28 \pm 1.7$ °C, pH = 8.1 ± 0.3, and CIP: $T = 28 \pm 1.2$ °C, pH = 7.9 ± 0.1).

The antibiotic concentrations in the experimental media of the toxicity experiments performed with ENR and CIP administrated in bath treatment increased gradually during the exposure period, up to 132 ± 20% (mean ± SD) of the nominal concentration for ENR, and up to 182 ± 86% (mean ± SD) of the nominal concentration for CIP on day 5. The antibiotic concentrations in the experimental media dropped to below LOD on day 14. CAT activities measured in fish exposed to ENR were not significantly different from the controls during the entire experimental period (Fig. 4A). CAT activity in fish muscle decreased significantly in the individuals exposed to 50 000 μg CIP/L on day 14 (Fig. 4B). Conversely, ChE activity in brain samples appeared to increase with increasing ENR and CIP water concentrations (Fig. 4C and D), showing a clearer dose–response effect in the experiment conducted with CIP. In the experiment conducted with ENR, a significant increase in the brain ChE activity was observed for the concentration of 10 000 and 800 μg of ENR $L^{-1}$ on days 3 and 14, respectively (Fig. 4C). In the experiment performed with CIP, the Kruskal–Wallis test indicated only marginally significant effects on day 5 ($p = 0.065$) and, therefore, the multiple comparison test could not be performed. Fishes exposed to 10 000 μg $L^{-1}$ of ENR showed an impaired swimming behaviour with no or very slow movements during the whole exposure period. These effects, however, were not longer noticeable 24 h after the antibiotic administration period. The measured temperature and pH values were: ENR: $T = 27 \pm 0.5$ °C, pH = 8.1 ± 0.4; CIP: $T = 28 \pm 1.0$ °C, pH = 7.8 ± 0.2.

**Table 1**
Results of the toxicity tests with enrofloxacin (ENR) and ciprofloxacin (CIP) on *Chlorella* sp. and *Moina macrocopa*, and water quality parameters measured during the toxicity experiments.

| Species | Antibiotic | Exposure duration | EC10 (μg $L^{-1}$) (95% CI) | EC50 (μg $L^{-1}$) (95% CI) | Temperature (°C) (mean ± SD) | pH (mean ± SD) |
|---|---|---|---|---|---|---|
| *Chlorella* sp. | ENR | 72 h | 3000 (4–14000) | 111000 (40000–281000) | 26 ± 1.8 | 9.1 ± 1.1 |
| | CIP | 72 h | 5200 (600–10000) | 23000 (13000–40000) | 25 ± 1.5 | 8.7 ± 1.2 |
| *Moina macrocopa* | ENR | 48 h | 41000 (30400–55000) | 69000 (57000–84000) | 32 ± 1.0 | 8.5 ± 0.2 |
| | CIP | 48 h | 50000 (36000–69000) | 71000 (61000–83000) | 33 ± 2.4 | 8.4 ± 0.3 |

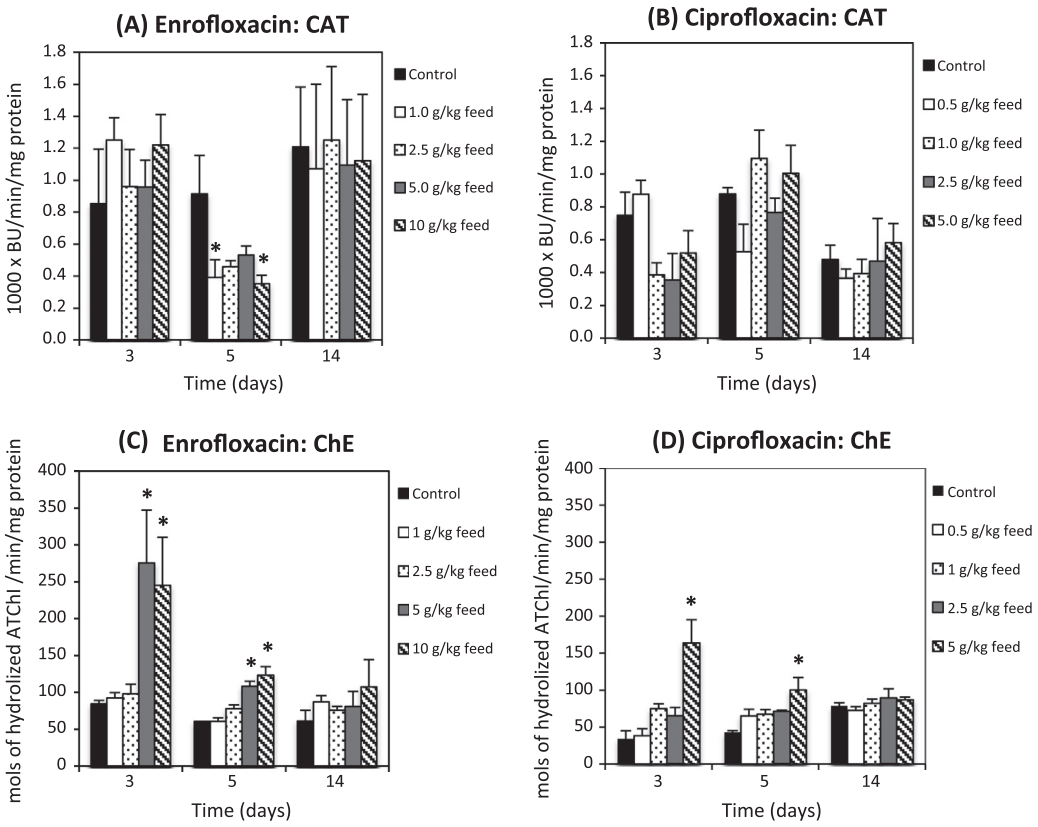


**Fig. 3.** Results of the catalase (CAT) and cholinesterase (ChE) activities (mean ± S.E.) in the toxicity experiments performed with enrofloxacin (A and C) and ciprofloxacin (B and D) administered via medicated feeds to *O. niloticus*.

### 3.3. Ecological risk assessment

The results of the risk assessment showed potential risks for cyanobacteria exposed to antibiotic concentrations in the water layer, with calculated RQs of 1.4 and 5.0 for ENR and CIP, respectively. Potential risks for cyanobacteria were also calculated for CIP exposure in sediments (RQ=9). The results of the risk assessment performed for algae, invertebrates and fish indicated insignificant risks (RQs < 1; Table 2).

## 4. Discussion

### 4.1. Antibiotic fate and exposure

To our knowledge, this is the first study that monitored antibiotic concentrations in freshwater aquaculture pond effluents during and after antibiotic medication. Based on the measured antibiotic concentrations in the pond effluents and the water exchange rates in the monitored pond, we estimated that about 18% of the applied ENR mass is discharged into the surrounding aquatic ecosystems (4.4% during the administration period and 13.7% during the 20 successive days), and 5.3% of the applied ENR applied mass is discharged in form of CIP. The estimated amount of ENR discharged into the environment corresponds fairly well with the modelling calculations performed by Rose and Pedersen (2005), who predicted that about 10–15% of the administered mass of the antibiotic oxytetracycline is released from fish hatcheries to the receiving water body during treatment and in the first 5 d thereafter.

Monitored ENR concentrations in the *Pangasius* catfish farm effluents are in the range of the antibiotic concentrations measured in livestock farm effluents, sewage treatment plant effluents and drainage systems of urban areas in the Mekong Delta (Managaki et al., 2007; Shimizu et al., 2013). ENR and CIP concentrations in the river sediments are in the range of the antibiotic concentrations measured by Rico et al. (2014b) in tropical river sediments

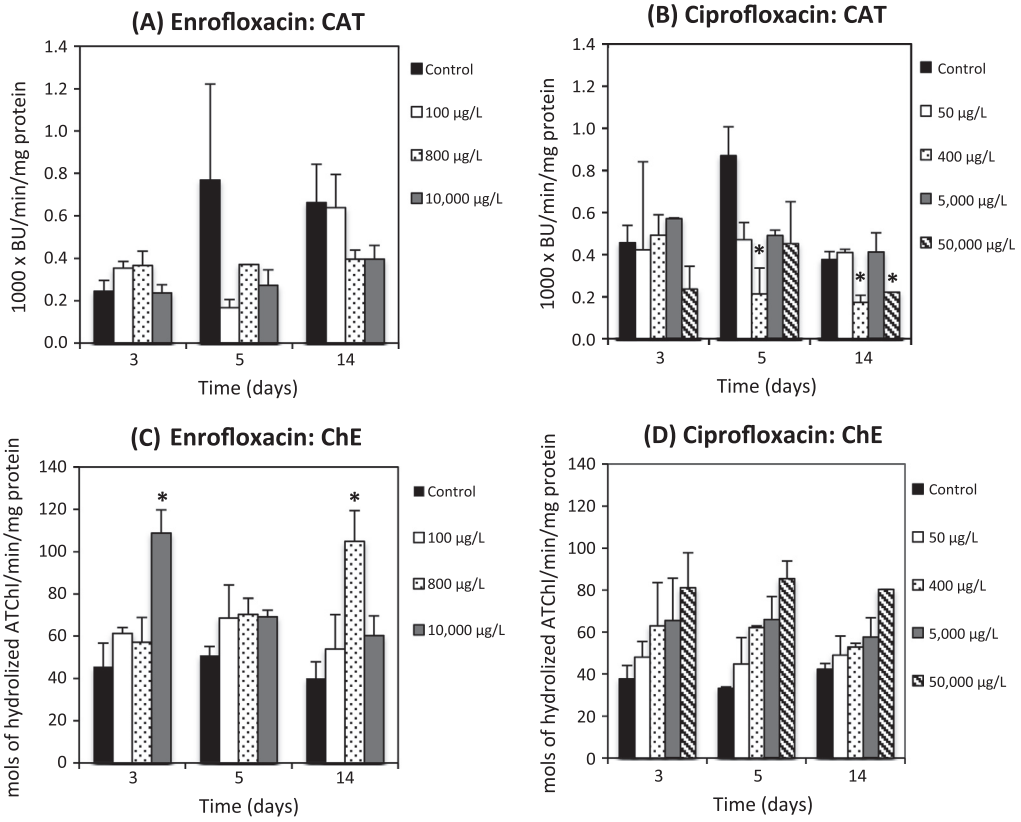


**Fig. 4.** Results of the catalase (CAT) and cholinesterase (ChE) activities (mean ± S.E.) in the toxicity experiments performed with enrofloxacin (A and C) and ciprofloxacin (B and D) administered in bath treatments to *O. niloticus*. The results of the highest ENR concentration (100 000 µg/L) are not displayed since all fish died before the third day after the start of the experiment. The standard error for the highest CIP concentration could not be calculated since all fish died in 2 out of the 3 replicates after day 5.

**Table 2**
Calculated risk quotients (RQ) for enrofloxacin (ENR) and ciprofloxacin (CIP).

| | Exposure concentrations | | | | | | Effect Assessment | | | | | | Risk Quotients | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ENR | | | CIP | | | ENR | | | CIP | | | ENR | | CIP | |
| | Water | | Sediment | Water | | Sediment | | | | | | | | | | |
| Taxonomic group | $C_{total}$ (µg $L^{-1}$) | $C_{diss}$ (µg $L^{-1}$) | $C_{pore\ water}$ (µg $L^{-1}$) | $C_{total}$ (µg $L^{-1}$) | $C_{diss}$ (µg $L^{-1}$) | $C_{pore\ water}$ (µg $L^{-1}$) | EC50 (µg $L^{-1}$) | AF | PNEC (µg $L^{-1}$) | EC50 (µg $L^{-1}$) | AF | PNEC (µg $L^{-1}$) | Water | Sediment | Water | Sediment |
| Cyanobacteria | | | | | | | 49.0[a] | 100 | 0.49 | 5.00[b] | 100 | 0.05 | 1.39 | 0.67 | 5.00 | 9.000 |
| Algae | 3.15 | 0.68 | 0.33 | 0.39 | 0.25 | 0.45 | 3100[a] | 100 | 31.0 | 2970[b] | 100 | 29.7 | 0.02 | 0.01 | 0.02 | 0.011 |
| Invertebrates | | | | | | | 53 300[c] | 1000 | 53.3 | 1200[c] | 1000 | 1.20 | 0.06 | 0.01 | 0.32 | 0.375 |
| Fish | | | | | | | 79 500[d] | 1000 | 79.5 | >60 000[e] | 1000 | >60 | 0.04 | <0.01 | <0.01 | <0.007 |

[a] Value for *Microcystis aeruginosa* (Robinson et al., 2005).
[b] Value for *Pseudokirchneriella subcapita* (Halling-Sørensen et al., 2000).
[c] Value for *Daphnia magna* (Kim et al., 2010).
[d] LC50 value for *Lepomis macrochirus* (Gagliano and Mc Namara, 1996).
[e] Value for *Gambusia holbrooki* (Martins et al., 2012).

of Thailand impacted by aquaculture pollution, and are comparable to other monitoring studies performed in other Asian rivers impacted by urban or agricultural pollution (e.g. Zhou et al., 2011; Xue et al., 2013). Our study showed that ENR and residual concentrations of CIP are released for periods up to several weeks after treatment, indicating that surrounding ecosystems are exposed to low-concentration antibiotic pulses for relatively long periods. In addition, our study demonstrated that antibiotic residues tend to accumulate in the sediments down-stream the effluent discharge point, where the water flow speed is reduced. This suggests that settling of organic particles are likely routes of transport and deposition of antibiotics into natural sediments, as previously proposed by modelling studies (e.g. Rose and Pedersen, 2005). Further research must be dedicated to evaluate the efficiency of waste-water treatment options such as the construction of decantation ponds or wetlands for the removal of antibiotic residues from *Pangasius* effluents, and should also evaluate the contribution of the eventual discharges of sludge from *Pangasius* ponds to the environmental contamination with antibiotics.

### 4.2. Antibiotic toxicity on aquatic organisms

#### 4.2.1. Antibiotic toxicity for Chlorella sp.

The EC50–72 h value for CIP calculated from our study results is very similar to the value calculated by Nie et al. (2007) for *Chlorella vulgaris* (EC50-96 h = 20 600 µg $L^{-1}$). Marked sensitivity differences between microalgae species to antibiotics have been reported (e.g. Robinson et al., 2005; Qin et al., 2011). The toxicity values for

*Chlorella* sp. calculated in this study are one or two orders of magnitude higher than those reported for cyanobacteria, and slightly higher than those reported for other green algae species (*Pseudokirchneriella subcapitata*) (Halling-Sørensen et al., 2000; Robinson et al., 2005). Therefore, this study supports the use of toxicity data for cyanobacteria to protect the structure of primary producer communities in antibiotic risk assessments. The higher tolerance of *Chlorella* sp. to antibiotics compared to other green algae species might be explained by the fact that their cell walls contain glucosamine polymers such as chitin and chitosan (Kapaun and Reisser, 1995), which could act as an extra permeability barrier, thus limiting antibiotic uptake (Bernard and Latgea, 2001).

#### 4.2.2. Antibiotic toxicity on M. macrocopa

Due to their high abundance and distribution in tropical aquatic ecosystems as well as their high sensitivity to toxicants, *Moina* sp. have been considered as good candidates for representing invertebrate communities and replacing testing with *Daphnia magna* in ecotoxicological assessments performed in tropical and subtropical regions (Daam et al., 2008). The calculated toxicity values for *M. macrocopa* in this study are in the order of the available toxicity values of ENR (EC50-48 h = 56 700 μg $L^{-1}$; Park and Choi, 2008) and CIP (EC50-48 h = 65 300 μg $L^{-1}$; Martins et al., 2012) for *D. magna*, suggesting no major sensitivity differences between the temperate and tropical cladocerans to the studied antibiotics. The EC50-48 h determined for ENR in the present study is more than two times lower than the one reported by Park and Choi (2008) for *M. macrocopa* (EC50-48 h > 200 000 μg $L^{-1}$; T = 25 ± 1 °C). This could be related to the higher temperature set in our tests (>5 °C difference), which could have enhanced chemical uptake (see Kim et al., 2010).

#### 4.2.3. Antibiotic toxicity on O. niloticus

Several studies have demonstrated that CAT and ChE activities are reliable biomarkers of antibiotic exposure in aquatic organisms (Table S2). Similarly to the results of the present study, Oliveira et al. (2013) measured a significant inhibition of CAT in zebra fishes exposed to oxytetracycline and amoxicillin via water. Our study was the first showing a trend towards an increase of ChE activity in brains of fishes exposed to fluoroquinolone antibiotics. This increase might be explained by the fact that fluoroquinolones can act as negative allosteric modulators and block or change the conformation of the orthosteric or allosteric binding sites of Acetylcholine (ACh) receptors, as reported in humans (Gregory et al., 2007). Therefore, all ACh released in the synaptic cleft would only bind to ChE such that its activity would increase. Moreover, fluoroquinolones could act as cholinergic agonists or modify the gene coding for choline acetyl-transferase, leading to an increase of ACh production (Rawi et al., 2011), which would be further hydrolysed by ChE resulting in a significant increase of the enzymatic activity.

In general, bath treatment application resulted in higher toxic effects than the oral administration method when considering the same applied dose (antibiotic weight per kg of fish). Milan et al. (2006) found that pharmaceuticals caused QT prolongation and cardiac disorders in zebra fishes, in a similar way that pharmaceutical overdoses affect humans. Therefore, according to the symptoms observed in our fishes exposed to the highest ENR and CIP water concentrations (i.e., muscle spasms and impaired movements until death), the (transient) failure of the cardiac system seems to be the most plausible cause of death and an important toxicity mechanism for these antibiotics in fishes.

In conclusion, the results of our fish biomarker experiments suggest that measured ChE activity in brain samples seems to be an appropriate biomarker for fluoroquinolone antibiotics and should be used in combination with CAT and Gluthation S-Transferase (Oliveira et al., 2013) in order to describe sub-lethal effects and physiological impairments in fishes treated with antibiotics at high dosages. However, the effective exposure concentrations calculated in this study (10 000 μg $L^{-1}$ for ENR and 400 μg $L^{-1}$ for CIP), and in other studies (Table S2), are one order of magnitude higher than the antibiotic concentrations measured in aquatic ecosystems polluted with aquaculture effluents. This suggests that the use of biomarkers for monitoring ecotoxicological effects of antibiotic pollution on wild fish populations has severe limitations, and should be restricted to the evaluation of the stress caused by antibiotics at the therapeutic doses used in aquaculture facilities. Furthermore, the symptoms and biomarker responses observed in the studied fishes seem to correspond fairly well with the available literature for humans, suggesting that the receptors and toxicity mechanisms of antibiotics might be somehow similar for different vertebrate species.

### 4.3. Ecological risk assessment

The results of the risk assessment performed in this study indicate that the environmental release of ENR and CIP residues after ENR medication in *Pangasius* catfish farms is posing minimal risks for algae, invertebrate and non-target fish communities. Only cyanobacteria and other bacterial taxa might be affected by antibiotic pollution. A microcosm study investigating the effects of enrofloxacin on tropical freshwater communities could not identify significant effects of this antibiotic on cyanobacteria species (Rico et al., 2014a). However, more research should be performed to better understand the toxicity of antibiotics on cyanobacteria communities and to assess potential side-effects on ecosystem structure, especially under tropical conditions. Since our study indicated that antibiotics tend to accumulate in sediments down-stream effluent discharge points, further assessments should be carried out by testing potential toxic effects of contaminated sediments on sediment dwelling organisms. In our study, we focused on one single antibiotic treatment, however *Pangasius* farms are often clustered around water sources and share water drainage systems. Therefore, more research should be dedicated to monitor the occurrence of residues of antibiotics from different groups in aquatic ecosystems surrounding *Pangasius* farms and to assess the ecological effects of antibiotic mixtures from different groups and antimicrobial modes of action. This information is of crucial importance for assessing the risks of antibiotics to tropical freshwater ecosystems and the sustainability of current antibiotic use practices in Vietnamese *Pangasius* production and in other intensive aquaculture species in Asia.

## 5. Conclusions

The results of our study indicate that the discharge of untreated effluents from *Pangasius* catfish farms should be considered as an important pathway of antibiotic pollution into the aquatic environment. The administration of ENR for treating bacterial diseases in *Pangasius* catfish farms is not likely to result in major risks for non-target aquatic organisms inhabiting water bodies receiving farm effluents. However, further investigations must be dedicated to assess potential consequences for microbial communities and associated ecological functions, as well as to evaluate the contribution of antibiotic residues to the development of antibiotic resistant bacteria in the environment. After the completion of this study, ENR and CIP were banned for use in Vietnamese aquaculture (VMARD, 2012) due to the significant number of international market rejections related to food safety alerts, and it is therefore expected that their sells and use have recently seen a significant

decline. However, given the large number of antibiotics that are currently used in *Pangasius* catfish production in the Mekong Delta region of Vietnam and the lack of regulations controlling their environmental discharge, further monitoring of aquaculture antibiotics in aquatic ecosystems and cost-effective methods for reducing their environmental discharge are urgently required.

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## Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chemosphere.2014.06.062.

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