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Multi-criteria approach for the environmental impact assessment of inland aquaculture

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Summary. Trout farming, that represents the most important sector for aquaculture inland production in Italy, can cause negative effects on aquatic ecosystems. Recently, in the framework of Water Frame Directive 2000/60/EC and national law DL 152/2006, concerning the sustainable uses of water resources, multi-criteria approaches have been suggested to evaluate the impact of fish farming on aquatic ecosystems. In this study trout farms of central Italy were selected to investigate the effects of their effluents, on receiving water bodies using a multi-criteria approach based on physico-chemical parameters, microbiological and macrobenthonic indicators, detected in sampling stations located upstream/downstream the trout farm. Moreover, antibiotic susceptibility against antibiotics allowed and/or forbidden by current law (D.lgs 193/56/06) was tested on *E. coli* strains. The results indicate variations of chemical parameters and biological indicators from upstream to downstream sites in some of the investigated farms. Antibiotic resistance of *E. coli* strains suggested a large use of tetracycline and a possible past use of chloramphenicol. This study represents a first contribute to the knowledge of fish farm impacts on aquatic systems in Central Italy.

Key words: inland aquaculture, multi-criteria approach, Water Frame Directive, 2000/60/CE, biological indicators.

Riassunto *(Approccio multi-livello per la valutazione dell'impatto ambientale dell'acquacoltura d'acqua dolce)*. La troticoltura rappresenta il settore più importante per la produzione ittica in Italia ed è in grado di causare effetti negativi sugli ecosistemi acquatici. Recentemente, dopo l'emanazione della Direttiva Europea 2000/60/CE sulla tutela delle acque, e il suo recepimento a livello nazionale con il DL 152/2006 riguardante gli usi sostenibili delle risorse idriche, è stata consigliata l'adozione di un approccio multi-livello nella valutazione dell'impatto causato dagli impianti di acquacoltura. dieci troticolture dell'Italia centrale sono state selezionate per esaminare gli effetti dei loro scarichi sugli ecosistemi acquatici, utilizzando un approccio multi-livello. Sono stati analizzati parametri fisici, chimici, indicatori microbiologici e i macroinvertebrati bentonici in stazioni di campionamento situate a monte e a valle degli impianti. La resistenza a tre antibiotici consentiti ed ad uno proibito dall'attuale legge (DL 193/56/06) è stata testata su ceppi di *E. coli.* I risultati ottenuti mostrano cambiamenti dei parametri chimici e degli indicatori biologici e microbiologici nei siti a valle di alcuni impianti. La resistenza agli antibiotici in ceppi di *E. coli* ha mostrato un ampio uso delle tetracicline e un possibile uso passato del cloramfenicolo. In conclusione, questo studio rappresenta un primo contribuito alla conoscenza degli impatti sui sistemi acquatici causati dagli impianti di troticoltura dell' Italia centrale.

Parole chiave: acquacoltura d'acqua dolce, approccio multi-livello, Direttiva quadro 2000/60/CE per la tutela delle acque, indicatori biologici.

INTRODUCTION

Aquaculture is defined as "the farming of aquatic organisms including fish, molluscs, crustaceans and aquatic plants" [1]. The worldwide demand for aquaculture products has constantly increased in the last decades [2]: it has been the fastest growing food sector for human feeding supply word-wide, with an average annual increase of 10%, since 1980.

The major aquatic species farmed in Europe are Atlantic salmon, carp and rainbow trout, which is the most important cultured finfish species, with a total production of 215 207 tons in 2003 followed by the Atlantic salmon with 162 585 tons [3].

European fish farm normally are small, geographically dispersed [4] and generally the trout producing sector is characterized by regionally rooted en-

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terprises with an average annual production of 100 tons per farm or less [5].

Italy followed the global trend, fish farm products increased from 160 000 tons in 1985 to 227 000 tons in 2000 [6] representing 34% of the national fish production. The most frequently farmed species is reared rainbow trout of North American origin. Indeed, trout farms represent the most important sector for the Italian fish production: they were mainly located in northern Italy in Friuli-Venezia Giulia, Veneto and Lombardia. In central Italy, these farms are spread along the Apennines, where rivers and streams are characterized by clear well oxygenated and cool waters, that make them suitable habitat for salmonid rearing.

The growth of some forms of aquaculture has demonstrated to generate negative externalities on environment and wild populations of aquatic organisms [6-11]. In particular fish farming has traditionally been shown to have adverse effects on the aquatic environment due to waste effluents [12-16] especially for the water bodies receiving effluents, enriched by wastes nutrients and drugs.

Sustainable uses of water resources and protection of aquatic ecosystem have become in prominence with the emanations of the European Water Frame Directive 2000/60/EC [17] and the corresponding Italian Decree DL152/2006 [18]. The Water Framework Directive (WFD) recognised to biological indicators a main role for the evaluation of ecosystem health. One of its major objectives is to achieve the good ecological status for all water bodies. Among biological elements required by WFD, benthic invertebrates are the most common organisms used for the monitoring of riverine ecosystems quality [19-22]. *Escherichia coli* and Enterococci were recommended as microbiological indicators, as required by the Italian DL 152/1999 [22]. These microorganisms are widely used to detect faecal contamination [23-25].

The use of veterinary medicines in aquaculture is not centrally regulated in the European Union, consequently each Member State has individually taken its own measures, to respond to the specific needs of the sector. In Italy, possession, use, prohibition and definition of veterinary medicinal products are defined by DL 193/56/06 [26], based on the previous EEC Regulation 2377/90 [27]. Before this Regulation was in force, antimicrobial drugs were massively used against numerous pathogens of fishes, leading to the appearance of antibiotic-resistant strains.

In this study a multi-criteria approach has been chosen to investigate the impact of fish farm effluents on aquatic ecosystem (*i.e.* streams and rivers) using physico-chemical water parameters, macrobenthic invertebrates communities and microbiological indicators as *E. coli* and Enterococci.

MATERIALS AND METHODS

Study area

Sampling stations were located, upstream and downstream watercourses near ten trout farms selected in the Apennines region (Central Italy). Three farms were placed near the Nera River, tributary of Tiber River, respectively at Castel Sant'Angelo (CSA), Ussita (USS) and Borgo Cerreto (CER); two farms discharge their effluents on Velino River, at Colli sul Velino (COL) and Canetra (CAN); other three farms were located on Aterno river Basin near Capestrano (CAP), Bussi sul Tirino (BUS) and Popoli (POP). One farm, Sefro (SEF), was selected on Scarcito River and one, Biselli (BIS), on Corno River.

Sampling methods and laboratory analysis

Physico-chemical, chemical water parameters, benthic fauna and microbiological indicators were investigated in two seasons, summer and winter 2006.

Physico-chemical and chemical water parameters

Physico-chemical water parameters: pH, conductivity and dissolved oxygen were measured in situ using WTW instruments.

Water samples, for chemical analysis, were taken in 1000 mL glass bottles and stored in the dark at 4 °C until analysis, that have been performed within 24 hour from sampling.

In laboratory nitrate (NO^{3-}), phosphate (PO_4^{3-}), chemical oxygen demand (COD), and ammonium (NH_4^+), were analysed using Merck Spectroquant kits and concentrations were spectrophotometrically determined.

Biological oxygen demand (BOD5) was determined using OxiTop method, based on pressure measurement in a closed system: microorganisms in the sample consume the oxygen and release CO2. Water samples were transferred into dark bottles with NaOH that absorbs CO2, which can be read directly in mg/L BOD5.

Benthic fauna

Sampling was performed using "kick sampling" method, according to the European standard [28]: macroinvertebrates were collected with a hand net (25-9-40 cm; mesh = 0.9 mm) from one bank to the other for 3 minutes, paying attention to cover all microhabitats present. Samples were preserved in 96% ethanol into plastic boxes. Benthic fauna identification was based on morphological characteristics [29, 30] and identified in the laboratory to the taxonomic level required to each group (*e.g.*, the genus for mayflies and stoneflies; the family for caddis flies and coleopterans). Extended biotic index method (Indice biotico esteso, IBE) [31] was applied to assess the water quality, based on benthic community data. The IBE value is specifically formulated to indicate levels of organic pollution in streams: it increases with the number of collected taxa and the presence of highly sensitive groups; values range between 0 (most degraded macroinvertebrate community) and 14 (most preserved community).

Community structures were evaluated using different indices: EPT (Ephemeroptera-Plecoptera-Tricoptera), based on relative abundances of most sensitive groups

Ephemeroptera-Plecoptera-Tricoptera [32]; 1-GOLD (1- Gasteropoda, Oligochaeta, Diptera), that takes into account the relative abundances of most tolerant groups, Gasteropoda, Oligochaeta, Diptera [33], Shannon index diversity based on both abundance and evenness of present species and numbers of families of benthic organisms found at each site. High values of these indexes indicate highly diversified and balanced communities.

Microbiological indicators

Water samples were picked up in sterile 250 mL flasks, stored at 4 °C and transferred to the laboratory. Samples were processed within 24 hours. Concentrations of *E. coli* and Enterococci, using the membrane filtration technique [34], were detected in order to investigate faecal pollution.

A serial dilution was performed on water samples, and an aliquot (10 mL) of each dilution was filtered on 0.45 µm membrane filters using a water vacuum pump. Membrane filters were placed onto plates, *Tryptone Bile X-Glucuronide* agar (TBX) and *Slanetz e Bartley* (SB), respectively for E.coli and Enterococci. TBX plates were incubated at 44 ± 0.5 °C for 24 h and SB plates at 37 ± 0.1 °C for 48 h.

Each experiment was performed in duplicate and the results were expressed as colony formation units (cfu/100 mL).

Antibiotic susceptibility

Until submitted to susceptibility test, each colony was cultured on to Tryptone Soya Agar (TSA).

Antibiotics susceptibility test was performed on all *E. coli* strains isolated from winter samples using Bauer-Kirby method [35]. Antibiotics tested against *E. coli* strains were: tetracycline, amoxicillin, flumequin and chloramphenicol. Susceptibility was determined by comparing zones of inhibition with reference values [36]. A negative control was also run (an aliquot of 0.5 McFarland culture on to Mueller Hinton Agar). Test and control plates were analyzed in duplicate and incubated at 37 °C for 24 h.

RESULTS

The results of physico-parameters, dissolved oxygen, pH and conductivity are showed below.

In the upstream sites: lowest concentration of dissolved oxygen has been found in BIS (4.45 mg/L) and the highest one in CSA (10.2 mg/L) in winter samples. The pH values had a range within 7.60 in CAN and 8.01 in CSA, in summer samples. Conductivity presented a range within 289 µS/cm in CSA and 860 µS/cm in CAN in summer samples.

In downstream sites: lowest concentration of dissolved oxygen has been detected in 4.35 mg/L in BIS, the highest in CSA (9.89 mg/L) in winter samples. The pH values had a range, in winter samples, within 7.40 of BIS and 8.05 of CSA. While conductivity presented a range within 290 µS/cm in CSA to 841 µS/cm in CAN in winter samples.

The results of chemical parameters: BOD5, COD, phosphates, nitrates and ammonium are reported below.

BOD5 concentrations increased from upstream to downstream in SEF in both seasons (from 1 mg/L to 11 mg/L) and in CAP (1 mg/L to 9 mg/L), in winter.

Concerning COD values detected, concentrations changed from upstream to downstream sites in summer in following trout farms: BIS (from 15.39 mg/L to 33.41 mg/L) and in USS (21.11 mg/L to 39.73 mg/L).

Increases in phosphate concentrations have been detected from upstream to downstream in SEF (from 0.045 mg/L to 0.33 mg/L) and in BIS (0.16 mg/L to 0.29 mg/L). Moreover high concentrations have been found, in summer samples, in following upstream sites: CSA (1.92 mg/L) and BUS (0.64 mg/L).

Nitrate concentrations increased from upstream to downstream in CAP (1.8 mg/L to 2.58 mg/L) in summer samples. Significant concentrations of nitrates

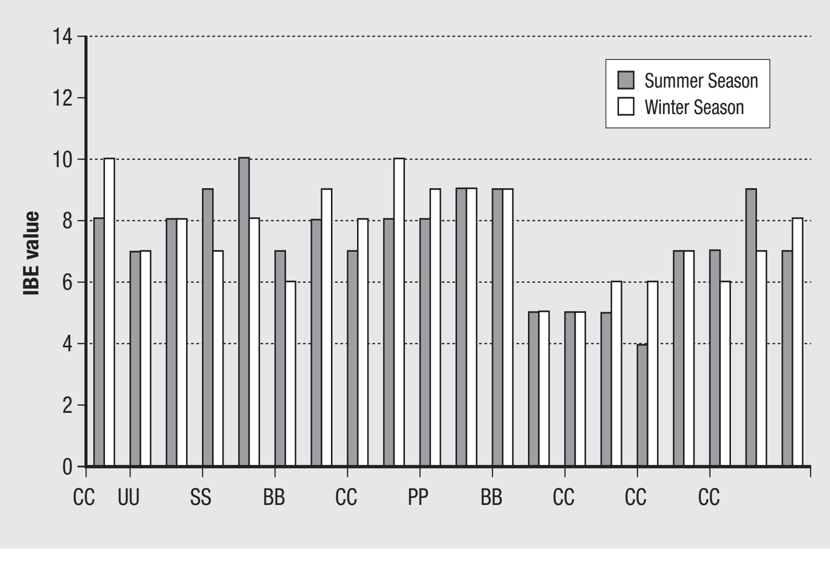

Fig. 1 | *Extended biotic index (IBE; Ghetti,1997) results in summer and winter samples.*
IBE value 0-4: Bad quality class;
IBE value 4-6: Poar quality class;
IBE value 6-8: Moderate quality class;
IBE value 8-10: Good quality class;
IBE value 10-14: High quality class.

have been detected in summer in BIS upstream and downstream (6.76 mg/L and 6.36 mg/L, respectively).

Ammonium concentrations increased in SEF from 0.067 mg/L in the upstream site to 0.97 mg/L downstream in winter. SEF upstream presented the high concentration (1.50 mg/L) also in summer samples.

A total of 15 780 organisms have been collected, 6178 during summer sampling and 9602 in winter sampling; 43 taxonomic groups were identified belonging to Plecoptera (9), Ephemeroptera (8), Tricoptera (8), Coleoptera (3), Diptera (7), Crustacea (2) and Gasteropoda (2) Hirudinea (2) e Oligochaeta (2).

The evaluation of biological quality relays on the analyses of benthic macroinvertebrate upstream and downstream of fish farms. Some differences between IBE index values were shown. In *Figure 1*, IBE application results has been reported. Slight variations between upstream and downstream values have been shown in BIS, CSA and CAN, where only one biological quality class shift was detected. IBE results showed also a worsening of two quality classes between the two SEF sites.

Results of EPT, 1-GOLD, Shannon index and number of families *(Table 1)* indicated a decrease from upstream to downstream only in SEF farm, where EPT value decreased from 0.30 to 0.11 and 1-GOLD from 0.72 to 0.2, Shannon index worsened from 2.06 to 1.3 and number of families reduced from 14 to 7. On the contrary, from upstream to downstream an improvement (increase) of EPT values has been detected in in USS and CAN, with values changed from 0.37 to 0.66 and from 0.23 to 0.63, respectively.

A seasonal variability was observed in microbiological results, except for CAN and SEF farms. In CAN upstream and downstream sites, high concentrations of *E. coli* (3×10^3 cfu/100 mL) and Enterococci (2×10^3 cfu/100 mL) were present in both sampling seasons.

In SEF upstream site, significant concentration of Enterococci (3×10^3 cfu/100 mL) has been found in both sampling seasons, whereas downstream, lower concentrations has been detected (1×10^2 cfu/100mL) in both sampling seasons.

In USS sites, the concentration of *E. coli* from summer to winter decreased from 3×10^3 to 4×10^2 cfu/100mL and of Enterococci from 1.2×10^3 cfu/100 mL to 2.5×10^2 cfu/100 mL.

In CSA concentration of *E. coli* changed from 2×10^3 to 4×10^2 cfu/100 mL from summer to winter. In CER Enterococci concentration ranged from 1.4×10^3 cfu/100 mL in summer season, to 1.4×10^2 cfu/100 mL in winter season.

All *E. coli* strains (n = 351) were tested against four antibiotics: 56% were sensitive and 44% were resistant *(Figure 2)*. The percentages of resistance to each antibiotic are the following: 28% to tetracycline, 3% to chloramphenicol, 2% to flumequine, 11% to amoxicillin. An increase of the resistant strains percentage was observed as a upstream-downstream gradient for tetracycline in CSA, USS and BIS samples.

Table 1 | *Macroinvertebrate indices EPT, 1-GOLD, Shannon index and number of families in summer and winter sampling*

Station Code	Summer sampling				Winter sampling			
	EPT	1-GOLD	Shannon index	Number of families	EPT	1-GOLD	Shannon index	Number of families
CSA UP	0.28	0.74	1.83	9	0.67	0.95	1.054045	11
CSA DOWN	0.57	0.70	2.12	13	0.46	0.55	1.666421	11
USS UP	0.44	0.57	2.32	12	0.61	0.72	1.51664	10
USS DOWN	0.50	0.62	2.08	11	0.37	0.82	2.008641	15
SEF UP	0.86	0.94	2.21	14	0.66	0.79	1.96945	13
SEF DOWN	0.58	0.65	1.86	9	0.32	0.72	2.061929	14
BIS UP	0.49	0.68	2.25	11	0.11	0.25	1.316015	7
BIS DOWN	0.50	0.70	1.94	10	0.79	0.83	1.895258	12
CER UP	0.67	0.89	1.83	10	0.63	0.79	2.052705	14
CER DOWN	0.31	0.64	2.17	10	0.76	0.89	2.333189	17
POP UP	0.44	0.90	1.72	12	0.82	0.89	1.893188	17
POP DOWN	0.44	0.76	2.20	12	0.20	0.93	1.252241	14
BUS UP	0.01	0.24	1.59	9	0.04	0.32	1.940123	10
BUS DOWN	0.01	0.34	1.81	9	0.10	0.60	1.739827	8
CAP UP	0.02	0.98	0.13	4	0.12	0.82	1.171751	9
CAP DOWN	0.02	0.87	0.60	7	0.04	0.91	0.61	7
COL UP	0.13	0.92	0.85	7	0.12	0.90	1.2	14
COL DOWN	0.11	0.93	0.96	12	0.09	0.89	1.03	9
CAN UP	0.74	0.86	1.73	12	0.23	0.458824	1.92	11
CAN DOWN	0.44	0.44	1.92	9	0.73	0.982972	1.45	12

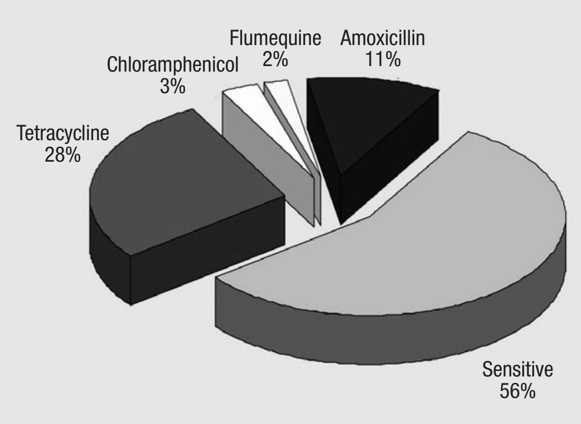

Fig. 2 | *Percentages of antibiotic susceptibility of* E. coli *tested strains.*

Furthermore, the same gradient was observed for chloramphenicol in CER samples.

DISCUSSION AND CONCLUSIONS

This multi-criteria approach, based on different biotic and abiotic indicators, aimed to investigate the variation of different parameters correlated to the impacts of aquaculture activities on river ecosystems.

Changes of physical parameters values from upstream to downstream sites have not been detected, whereas an increase of chemical values has been pointed out, especially in the waste receiving stretches of rivers fir the SEF, CAP and BUS sites, in agreement with previous studies conducted in the trout farm in BUS [37, 38]. These results suggested the negative externalities of the fish farm wastewaters on river water quality [39-41].

Concerning the benthic communities, IBE results showed a slight negative effects on biological quality of lotic systems generated by fish farms waste water discharges, in BIS, CSA and CAN. Moreover this index emphasized the impact of SEF farm on the river ecosystems, as confirmed also by the EPT, 1-GOLD, Shannon index and number of family values obtained in these sites. A peculiar situation was obtained in CAN, where IBE indicated a worsening from upstream to downstream, whereas the other indices suggested an improving of macrobenthic community between the two sites. The increased value of EPT and 1-GOLD from upstream to downstream stations and the similar values of Shannon index and number of families in both sites indicated generally a lack of effect of the trout plants on these biological communities.

IBE values classified in poor biological quality class BUS and CAP upstream and downstream sites, and in moderate quality class both stations of COL. These results suggested that other anthropogenic impacts such as alteration of hydro-morphological characteristics, pollution from agriculture and other discharges appeared more important in influencing biological quality of lotic ecosystems where trout farms are located.

Microbiological results suggested the presence of isolated and point discharges, except for the contamination from both *E. coli* and Enterococci detected in SEF, CAN and CSA, which was probably due to widespread livestock activities.

The comparable levels of resistance found among bacteria isolated upstream and downstream the fish-farms, indicated a correct use of amoxicillin and tetracycline. Furthermore, the presence of chloroamphenicol resistant strains downstream CER farm may be explained by the massive past use of this antibiotic.

In conclusion, this study aimed at giving a contribute to the improvement of the knowledge on environmental impacts of inland aquaculture, in the region of central Italian Apennines, showing a relative environmental sustainability of these activities, with low negative externalities to aquatic ecosystems.

Moreover, an ecosystem approach to the intensive aquaculture in inland water should be applied to reorient this sector towards sustainability [42], *i.e.* adopting the guidelines of "Code of conduct for responsible fisheries"[43], "Code of conduct of European aquaculture" the Commission Regulation (EC) 710/2009 concerning organic aquaculture [44] and other management protocols for "environmental friendly aquaculture" [45].

Conflict of interest statement

There are no potential conflicts of interest or any financial or personal relationships with other people or organizations that could inappropriately bias conduct and findings of this study.

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