

## Ecopathology in aquaculture: risk factors in infectious disease outbreak \*

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**Summary** — This paper describes a study of the risk factors associated with disease outbreaks in fish species of fish farms and rivers of north-east Spain. We focused our work on the isolation of fish pathogens (bacteria, virus), the water quality (physicochemical and microbiological quality) and management characteristics. We have observed 2 important viral diseases, infectious pancreatic necrosis and spring viraemia of carp, and 2 important bacterial ones, furunculosis (*Aeromonas salmonicida*) and bacterial kidney disease (BKD) (*Renibacterium salmoninarum*). Our preliminary results show that there are some potential risk factors associated with the main diseases of fish, such as fish age, fish species, production system, season and water temperature, but their role depends on the disease.

**aquaculture / ecopathology / risk factor / fish / pathogen / environment**

**Résumé** — **Écopathologie en aquaculture. Facteurs de risque des maladies infectieuses.** *Les facteurs de risque associés à l'apparition de maladies chez les espèces de poissons d'élevage ou sauvages dans le nord-est de l'Espagne ont été étudiés. Les agents pathogènes (bactéries, virus) ont été isolés à partir des poissons, la qualité physico-chimique de l'eau et le type d'exploitation ont été aussi étudiés. Nous avons observé la présence de 2 maladies virales importantes, la nécrose pancréatique infectieuse et la virémie printanière de la carpe, et 2 maladies bactériennes importantes, la furonculose (*Aeromonas salmonicida*) et la rénibactériose (*Renibacterium salmoninarum*). Les résultats préliminaires sur l'étude des facteurs de risque ont montré une association entre quelques facteurs et les principales*

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*maladies des poissons. Ces facteurs étaient : l'âge du poisson, l'espèce du poisson, le système de production, la saison et la température de l'eau ; mais le rôle de ces facteurs de risque observés était différent pour chaque maladie.*

**aquaculture / écopathologie / facteur de risque / poisson / pathogène / environnement**

## INTRODUCTION

High morbidity and mortality rates produced in the affected population are the most important ecological and economic consequences of viral diseases in aquaculture (McAllister, 1988; De Kinkelin *et al*, 1991). The main diseases affecting fish intensive production and/or wildlife fish are viral diseases, viral haemorrhagic septicaemia (VHS), infectious haematopoietic necrosis (IHN), infectious pancreatic necrosis (IPN) and spring viraemia of carp (SVC); and bacterial diseases, furunculosis, bacterial kidney disease (BKD), red mouth (*Yersinia ruckeri*), and pasteurellosis (*Pasteurella piscicida*) (Bullock *et al*, 1976; McAllister, 1988; De Kinkelin *et al*, 1991). These aetiological agents are influenced by factors independent of the fish and the agent, such as environment, production system factors, nutrition, and others that play an important role as risk factors of the disease outbreak (Tufféry, 1977; Enzmann *et al*, 1992). The significance of these risk factors convinced us to carry out a study to define the health status and investigate the role of some risk factors predisposing disease outbreak in the intensive and wildlife aquaculture of Spain. The final aim of our study was to orientate fish intensive production and river repopulation according to these risk factors and the sanitary level of the different areas. The results of this paper are preliminary, and will be completed in the future.

## MATERIALS AND METHODS

This study involves a 4-year survey (1993–1996) in fish farms and rivers located in north-east of

Spain (Aragón and Navarra regions). The samplings were made every 3 months in 3 chosen pools of every fish farm. The studied fish species were rainbow trout (*Oncorhynchus mykiss*), brown trout (*Salmo trutta*), Atlantic salmon (*Salmo salar*), carp (*Cyprinus carpio*), nase (*Chondrostoma toxostoma*), gudgeon (*Gobio gobio*) and barb (*Barbus graellsii*). There were 4 levels in our study.

## Epidemiological survey and data-base design

The epidemiological survey was designed according to the factors suspected of playing a role in fish health, using ecopathological concepts and principles (Martin *et al*, 1987; Thrusfield, 1990). This survey consists of the following parts: fish characteristics: age, sex, species, weight, size and physiological status; environmental information: water speed, algae, and muddiness; water quality: physicochemical and microbiological quality of water; management information: production cycles, raceway density, type of pools, treatments and desinfection systems; and isolated agents: bacterial, viral, mycotic and parasitic agents.

This information was introduced into a data base, designed within the epidemiological computer program EPI-INFO (WHO).

## Infectious disease diagnosis

The fish assemblage in every sampling was determined with the tables of sample size (Ossiander and Wedemeyer, 1973; Simon and Schill, 1974). We concluded that it was necessary to take 15 fish from 3 different pools of the fish farms, and 10 fish from 2 points of the rivers, 1 site upstream and another downstream from the fish farm.

On the sampled animals, we performed a necropsy and took samples of the organs. Liver, spleen and kidney were cultured on bacterial media, tryptic soy agar, brain heart infusion agar, marine agar, blood agar, cytophaga agar, and

KDM-C agar (*Renibacterium salmoninarum* isolation medium) (Austin, 1985). Isolated bacteria were identified by classical staining, biochemical and growing tests.

Samples from liver, spleen, kidney, brain, piloric caeca and gonads, were inoculated in cell culture lines, bluegill fibroblast (BF-2), chinook salmon embryo (CHSE-214) and epithelioma papulosum cyprini (EPC), looking for viral agents (Wolf, 1988, EEC, 1992).

**Water quality tests**

Samples of water were taken to determine its physicochemical and microbiological quality from every site in the fish farms and rivers, upstream as well as downstream. Fourteen variables were measured: water temperature, pH, oxygen concentration, redox potential, conductivity, alkalinity, turbidity, hardness, ammonium, nitrate, nitrite, CO<sub>2</sub>, phosphate and heavy metal concentrations (Stirling *et al*, 1985).

**Data analysis**

The information obtained previously was entered into the data base. The analysis was performed with the computer programs EPI-INFO (Analysis and Statview-Se Graphics, using the statistical parameters chi-square distribution, Student's *t* distribution and correlation coefficient, depending on the type of variable (statistical association means  $P \leq 0.05$ ).

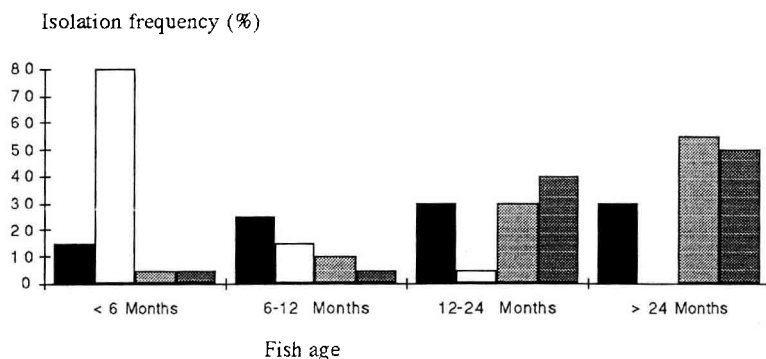
**RESULTS**

The aetiological agents reported as being the most frequent pathogens during the first year of the study are indicated in table I. We have observed that the most frequent bacterial disease, furunculosis, was located in the north-west of the study area, while the viral diseases were spread in the south-east of the geographical area of the study.

On the other hand, we have studied the risk factors associated with the isolated

**Table I.** Isolated aetiological agents and their frequency.

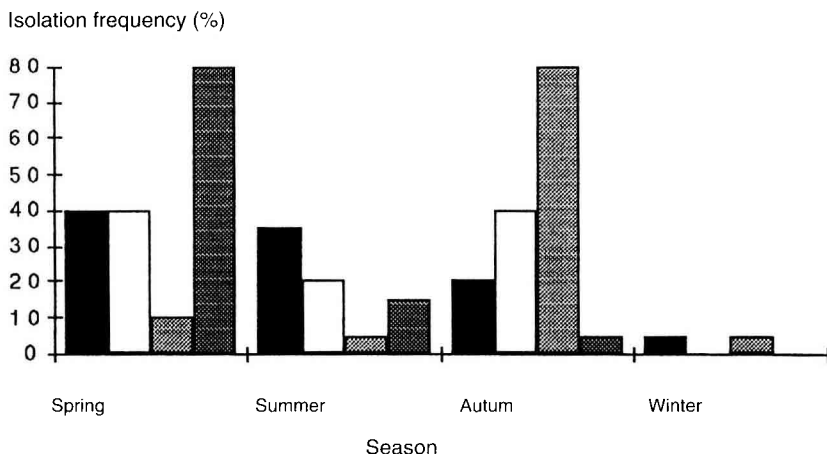
Agent	Frequency (%)
<i>Aeromonas salmonicida</i>	18.8
<i>Renibacterium salmoninarum</i>	14.2
<i>Aeromonas</i> spp (motile)	31.1
<i>Flavobacterium</i> spp	10.5
Infectious pancreatic necrosis virus	7.5
Spring viraemia of carp virus	1.3



**Fig 1.** Role of age as risk factor in the most important detected diseases: ■ furunculosis; □ bacterial kidney disease (BKD); ▒ infectious pancreatic necrosis (IPN); ■ spring viraemia of carp (SVC).

**Table II.** Isolation of *Aeromonas salmonicida* (furunculosis), *Renibacterium salmoninarum* (BKD), infectious pancreatic necrosis virus (IPN), and spring viraemia of carp virus (SVC), and their associated risk factors (statistical association).

Associated factors	Pathogens			
	<i>A. salmonicida</i>	<i>BKD</i>	<i>IPN</i>	<i>SVC</i>
Fish age	> 6 months	> 1 year	< 1 year	> 1 year
Fish species	Rainbow trout Brown trout	Rainbow trout Atlantic salmon Nase	Rainbow trout Nase, Gudgeon	Carp
Production system	Fish farming	Fish farming	Fish farming	Wildlife fish
Season	Spring Summer	Autumn	Spring Autumn	Spring Summer
Water temperature	> 16°C	12–18°C	8–15°C	16–22°C



**Fig 2.** Role of season as risk factor in the most important detected diseases: ■ furunculosis ; ▨ bacterial kidney disease (BKD); □ infectious pancreatic necrosis (IPN); ▩ spring viraemia of carp (SVC).

agents. We observed a statistically significant relationship between pathogen isolation and the following risk factors: fish age, fish species, production system, season and water temperature. The role these factors played differed depending on the disease (table II; figs 1, 2).

## DISCUSSION

### *Principal diseases detected*

The study of the sanitary state of the aquaculture of north-east Spain showed that

furunculosis, BKD, IPN and SVC were the most frequent diseases in the studied area, a result that is similar to those observed by other authors in a screening in the regions of Aragón and Galicia (Ortega, 1991; Bandin *et al*, 1992) which detected *Aeromonas salmonicida* and IPN virus as the principal pathogens.

### **Risk factors associated with disease outbreak**

The observed results presented in table II showed that there were some important associations to consider.

Furunculosis, BKD and IPN were related to intensive production of rainbow trout, brown trout, Atlantic salmon, and other fish species living upstream and downstream from the fish farms, nase and gudgeon. SVC was only associated with wild carp due to the absence of carp fish farms of intensive production. Our results agree with McAllister (1988) and De Kinkelin *et al* (1991), who maintain that intensive production is an important risk factor in outbreaks of fish diseases.

Season and water temperature were 2 important risk factors that seemed to be associated with disease outbreak. In general, we can conclude that viral diseases were associated with low temperatures, and bacterial diseases with high ones, except for SVC (viral disease), which was more frequent in high temperatures, because carps live in warmer water than salmonids. The same results were observed in previous studies in the region of Aragón (Ortega, 1991).

The association between disease outbreak and temperature involves an indirect association between season and disease. We could therefore detect bacterial diseases during seasons with high temperatures, and viral diseases during seasons of low tem-

peratures. Nevertheless, the most important periods of risk are the seasons when important changes in temperature take place, *ie* spring and autumn. This situation produces stress and immunosuppression of the fish defense mechanisms, as observed Van Muiswinkel *et al* (1985) in different fish species.

The age of the fish acted as another important risk factor in disease outbreak. We observed that IPN was detected in animals younger than 6 months old, whereas furunculosis was associated with animals older than 6 months old; BKD and SVC were associated with animals older than 1 year. In contrast to our results, some authors observed SVC in animals of all ages, including younger than 6 months old (McAllister, 1988; Wolf, 1988).

The fish species affected by the different diseases is the last risk factor we will discuss. We only observed SVC in carp, which means that the rest of the studied species were not sensitive to the disease. Furunculosis was mainly detected in brown trout, which confirms it as one of the most sensitive fish species, together with rainbow trout. Therefore, brown trout can act as an asymptomatic carrier in rivers where fish farms are located maintaining the infection for long periods of time.

Upstream and downstream from rainbow trout farms in which IPN virus was detected, we could isolate this pathogen from nase and gudgeon, fish species living in the rivers. This means that these species act as asymptomatic carriers of the virus, as observed in previous studies (Ortega, 1991).

BKD was detected in rainbow trout and Atlantic salmon, which were the principal sensitive species (Bandin *et al*, 1992). It was not observed in nase, which could be an important asymptomatic carrier in rivers where susceptible salmonids are located.

Our preliminary results allow us to conclude that disease outbreaks in northeast

Spain are conditioned by some common risk factors, which play a different role depending on the disease. We are presently continuing our analysis of other suspected risk factors and their association with disease outbreak in the aquaculture of north-east Spain.

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## REFERENCES

- Austin B (1985) Evaluation of antimicrobial compounds for the control of bacterial kidney disease in rainbow trout. *J Fish Dis* 8, 209-220
- Bandin I, Santos Y, Nuñez AE, Toranzo A, Barja JL (1992) BKD in Galicia (NW Spain): first isolation of *Renibacterium salmoninarum*. *Bull Eur Ass Fish Pathol* 12, 196-197
- Bullock GL, Rucker RP, Amend D, Wolf K, Struckey HM (1976) Infectious pancreatic necrosis: transmission with iodine-treated and non-treated eggs of brook trout (*Salvelinus fontinalis*). *J Fish Res Board Can* 33, 1197-1198
- Commission of the European Communities (1992) Proposal for a council regulation (EEC) concerning the animal health conditions governing the placing on market of aquaculture animals and products (Com 92/458 final). 16 November, Brussels, Belgium
- De Kinkelin P, Michel C, Ghittino P (1991) *Tratado de las enfermedades de los peces*. Acribia, Zaragoza, Spain
- Enzman PJ, Konrad M, Rapp J (1992) Epizootiological studies on viral haemorrhagic septicaemia in brown trout *Salmo trutta* Fario. *Dis Aquat Org* 12, 143-146
- Martin SW, Meek AH, Willeberg P (1987) Theoretical epidemiology: systems analysis and modeling. In: *Veterinary Epidemiology, Principles and Methods* (SW Martin, ed) Iowa State University Press, Ames, IA, USA, 193-218
- McAllister PE (1988) Infecciones víricas de peces cultivados. In: *Patología en acuicultura* (M Prensa, ed) Madrid, Spain, 37-158
- Ortega C (1991) Papel de diversas especies icticas no naturalmente sensibles a la Necrosis Pancreatica Infecciosa (IPN) que habitan los rios aragoneses como portadores del virus IPN, y su detección mediante diferentes técnicas diagnosticas. Doctoral Thesis, Facultad de Veterinaria, Universidad de Zaragoza, Spain
- Ossiander FJ, Wedemeyer G (1973) Computer program for sample sizes required to determine disease incidence in fish populations. *J Fish Res Board Can* 30, 1383-1385
- Simon RG, Schill WB (1974) Tables of samples size requirements for detection of fish infected by pathogens: three confidence levels for different infection prevalence and various population size. *J Fish Dis* 7, 515-520
- Stirling HP, Beveridge MCM, Ross LG, Philips MJ (1985) Chemical and biological methods of water analysis for aquaculturalists. Institute of aquaculture, University of Stirling, UK, 69-85
- Thrusfield M (1990) Encuestas. In: *Epidemiologia Veterinaria*, Acribia, Zaragoza, Spain, 191-205
- Tufféry G (1977) Recherches sur la Bucéphalose à *Bucephalus polymorufus* Baer 1827. Introduction à l'écopathologie des systèmes piscicoles. Thèse pour le diplômes de Docteurs de 3e cycle, université de Paris VI, France
- Van Muiswinkel WB, Anderson DP, Lamers CHJ, Egberts E, Van Loon JJA, Ijssel JP (1985) Fish Immunology and fish health. In: *Fish Immunology* (MJ Manning, ed) Academic Press, London, UK 1-8
- Wolf K (1988) *Fish viruses and fish viral diseases*. Cornell University Press, Ithaca, NJ, USA