# A CORRELATION BETWEEN ALKALINE PHOSPHATASE AND PHOSPHATE LEVELS WITH THE BIOMASS OF TROUT FARM EFFLUENTS

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## KORELACIJA IZMEĐU NIVOA ALKALNE FOSFATAZE I FOSFATA U OTPADNOJ VODI RIBNJAKA SA MASOM PASTRMKI

***Apstrakt***

Otpadne vode iz pastrmskih ribnjaka se obično ispuštaju u nezagađene akvatične ekosisteme, s obzirom na to da su ribnjaci najčešće locirani na velikim visinama, gde nema drugog izvora antropogenog zagađenja. Cilj ove studije je da se istraži odnos između aktivnosti alkalne fosfataze (APH) izmerene u otpadnim vodama i mase riba u ribnjaku. Uzorci vode sa ribnjaka su prikupljani u proleće i leto i iz nje su određivani nivoi APH, fosfata ($PO_4$-P) i količine bakterija (CFU). Ustanovljena je pozitivna korelacija između mase riba i nivoa APH i $PO_4$-P u efluentu, dok sa druge strane, nije utvrđena korelacija između mase riba i CFU. Rezultati ove studije su pokazali da nivo APH može biti korišćen kao potencijalni indikator zagađenja vodenih ekosistema otpadnih voda iz pastrmskih ribnjaka.

*Ključne reči: Pastrmski ribnjak, APH, bioindikacija, rečna ekologija*
*Keywords: trout farm, APH, bioindicator, rivers ecology*

## INTRODUCTION

Fish farming often results in a generation of effluents with high nutrient load with adverse effects on both the water quality and the aquatic ecosystem downstream. Fresh water Aquaculture activity of salmonids is frequently located by rivers at high altitudes. These aquatic ecosystems are frequently characterized by low pollution from agricultural or industrial activities and offer optimal temperature and water quality conditions for the farming of fish. A useful indicator of aquatic anthropogenic pollution is the bacterial load of aquatic ecosystems (Ackerman and Weisberg, 2003). At high altitudes and upstream of rivers, aquaculture may be the single anthropogenic source of aquatic pollution. Under intensive aquaculture conditions, the use of antibiotics is frequent and the high organic and bacterial load of aquaculture effluents may result in increased bacterial load downstream with antibiotic resistant strains (Gordon *et al.,* 2007). The effluents of land-based fish farms may vary in nutrient load according to the aquaculture management. For example, some farms may have effluent treatments of varying efficacy prior to discharge while others may vary in size and feeding regimes and thus use larger volumes of water and discharge more or less dilute waste (Rosenthal, 1994; Bergheim and Brinker, 2003).When fish farms are located upstream in river zones with no other human activity, the pollution generated by aquaculture can be easily assessed by monitoring environmental conditions upstream and downstream. Several chemical and biological indicators can be used to monitor the ecological status of a river (Camargo *et al.,* 2011). Frequently used chemical indicators include: pH, BOD, oxygen, nitrates, nitrites, phosphorus and ammonia levels (Tello *et al.,* 2010). Any possible alteration of these parameters between upstream and downstream river water of a fish farm can be attributed to the aquaculture activity. An additional index of fish farm effluents in river waters is the presence of extracellular enzymes released by the growing fish and the bacterial load of fish tanks. For example, Alkaline phosphatase (APH) activity in sediments downstream of fish farms may indicate higher bacterial load generated by the fish farm pollution (Baldock and Sleigh, 1988) but an additional source of APH is the excretion of farmed fish which can persist in fish farm effluents even after a septic tank treatment (Carr and Goulder, 1990). As a result, elevated levels of APH in rivers downstream of a fish farm is a combination of bacterial APH and excretion of farmed fish.

The aim of the present study was to investigate the hypothesis that the levels of free APH activity present in the outflow of fish ponds correlated with the biomass of the fish. This possible relationship would offer a tool to assess the aquaculture impact on streams and the potential capacity of fish farms to generate APH. This enzyme could under some circumstances contribute to elevated hydrolysis of organic phosphates and thus result in higher levels of inorganic $PO_4$ and accelerate eutrophication (Carr and Goulder, 1990).

## MATERIALS AND METHODS

The study was carried out between May and June of 2010. Water samples were collected in sterilized glass bottles, at the outflows of fish tanks of a commercial rainbow trout fish farm in NW Greece (using a concrete raceways flow-through system of rearing).

The water samples were collected between 9:00 and 11:00 hours to measure phosphates ($PO_4$-P) according to APHA (2005). The determination of bacteria was carried out with the filtration technique and using agar plates, in triplicates. They were then incubated for 24h at 30°C. Alkaline phosphatase activity was assayed according to Cao et al. (2005). Triplicates of 5ml water samples were supplied with Tris-HCl buffer (pH 8.5, final concentration 13 mmol $L^{-1}$), $Na_3N$ (final concentration 5mmol $L^{-1}$), and p-nitrophenyl phosphate (pNPP, final concentration 0.3 mmol $L^{-1}$), and the samples were incubated at 37°C for 12 h. The absorbance of p-nitrophenol was measured spectrophotometrically at 410 nm. APH activity was calculated as μmoles of p-nitrophenol released $L^{-1}$ $day^{-1}$. The statistical significance of the regression between fish biomass, bacterial load, phosphorus and the Alkaline phosphatase activity was determined using ANOVA ($P<0.05$).

## RESULTS

The biomass of fish in each tank ranged from 200 to 590 kg. The concentration of phosphorus in the effluents of trout farm tanks ranged between 0.13 to 0.43 mg $L^{-1}$ (Fig. 1). There was a significant relationship between fish biomass and phosphorus load of the pond effluents ($R^2=0.22$, $F=5.36$, $P<0.05$). The levels of APH in the effluent of each tank ranged between 0.60 and 2.34 μmoles of p-nitrophenol $L^{-1}$ $day^{-1}$ (Fig. 2). There was a significant relationship between fish biomass and phosphorus load of the pond effluents ($R^2=0.30$, F=7,81, $P<0.05$).

The bacterial load (CFU $10^6$ $mL^{-1}$) in the effluents of trout farm ponds with different biomass ranged between 0.08 to 1.40 (Fig. 3). There was no significant relationship between fish biomass and CFU in the effluents. Body mass correlated significantly with levels of APH and $PO_4$-P levels in the effluents. There was no significant relationship between fish biomass and CFU in the effluents.

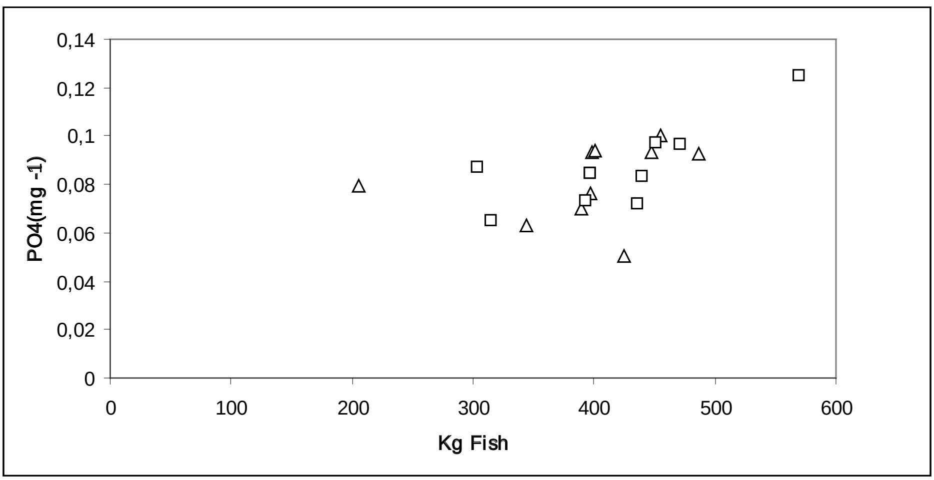


**Figure 1.** The concentration of phosphorus in the effluents of trout farm tanks with different biomass. Samples were collected in May (triangles) and June (squares) 2010.

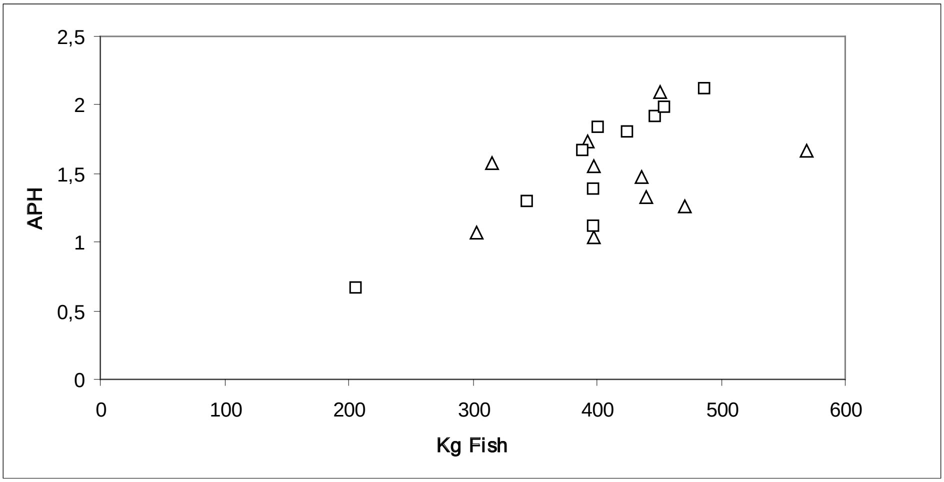


**Figure 2.** The activity of Alkaline Phosphatase (μmoles of p-nitrophenol $L^{-1}$ $day^{-1}$) in the effluents of trout farm ponds with different biomass. During two different sampling sessions: May (triangles), June (squares).

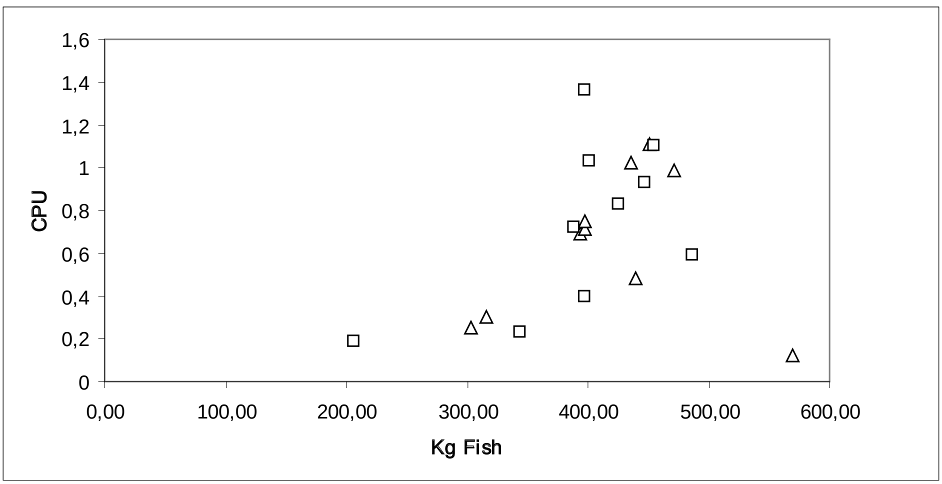


**Figure 3**. The bacterial load (CFU $10^6$ $mL^{-1}$) in the effluents of trout farm ponds with different biomass. Samples were collected in May (triangles) and June (squares) 2010.

## DISCUSSION

Both the activity of APH and the release of $PO_4$-P increased significantly with fish biomass. The activity of APH in the unfiltered effluents can be attributed to the combination of fish and bacteria present in the tanks whereas in the filtered water APH activity accurately represents the enzyme generated by the farmed fish (Carr and Goulder, 1990). The observed results support the relationship between fish tank biomass and APH activity in the effluents.

The $PO_4$-P released in fish tanks originates from fish feeds and excretion and can vary according to fish biomass and fish feed composition and feeding regimes (Satoh et al., 2003). There was no significant relationship between the bacterial load and fish biomass. The bacterial load of fish farm effluents stems from dissolved organic matter derived from uneaten feed and fish faeces as well as sediment flora (Bedwell and Goulder, 1997). Uneaten feed and faecal excretions of fish can vary in tanks according to the feeding rate regime, but sediment flora can vary according to tank conditions and cleaning procedures (Sindilariu, 2007).

According to standard aquaculture practices, during the growing season of trout farms, feed input in ponds varies according to fish biomass. The relationship observed in this study between the fish biomass and the bacterial and $PO_4$-P loads of fish pond effluents reflects a relationship between feeding rate and the potential environmental impact of land based fish farms (Boaventura et al., 1997). In several cases, even when river fish farm effluent are treated prior to discharge, the phosphates and microbiological parameters are significantly increased downstream from the fish farm (Ruiz-Zarzuela et al., 2009) with potential consequences for the aquatic ecosystem downstream of farm sites (Tello et al., 2010; Waring et al., 2012). The microbial load in the effluent may include antibiotic resistant strains with consequences for the public health (Naviner et al., 2011). The treatment of aquaculture water effluent, for example in settlement ponds for aquaculture water effluents, can reduce the organic load and microbial load (Snow et al., 2012) but regular pond management procedures such as fish harvesting and pond cleaning may result in sudden peaks of organic and microbial load of the fish farm effluents (Hinshaw and Fornshell, 2002).

The results of the present study indicate that the biomass of trout stocked in each tank correlated significantly with levels of APH and $PO_4$-P levels in the effluents but there was no correlation between fish biomass and the microbial load. The monitoring of APH levels could be used as a potential biomarker of fish farm pollution in the aquatic ecosystem.

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