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## ASSESSING TROUT FARMING IMPACTS ON RECEIVING WATERCOURSES: SELECTION OF THE MOST EFFECTIVE MACROINVERTEBRATE AND DIATOM METRICS

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### Introduction

Mountain aquatic ecosystems, due to their oligotrophic characteristics, are particularly sensitive to human activities. Inland trout farming can affect receiving watercourses by increasing nutrients (especially nitrogen and phosphorous compounds), organic matter and suspended solids, as well as through the release of drugs and chemicals. The extent of these impacts depends on several factors, such as farm size, the volume of water diverted, and the self-purification capacity of downstream waterbody. Furthermore, these effects can vary spatially and temporally, influenced by farming practices, stocking densities, feed quality and hydrological conditions. Local impacts are typically observed up to a kilometer downstream of the trout farm, but occasionally, they can extend to a regional scale.

In the Province of Trento, trout farming constitutes a significant production sector with approximately 60 trout farms mainly focused on the rearing of rainbow trout (*Oncorhynchus mykiss*). In this context, the project “Filiera ASTRO: Competitiveness and Sustainability of Mountain Aquaculture” (CUP J38H23001450007), funded by “Piano Nazionale Complementare – PNC” of the Ministry of Agriculture, Food Sovereignty and Forests (MASAF), aims to improve the local aquaculture industry through the reuse of fish farming by-products, and applying new technologies to reduce feed waste and environmental impacts. The project framework consists of four work packages (WP).

The main objectives of WP2 (Environmental sustainability of aquaculture and water courses quality) are: (I) assess specific aquaculture impacts on receiving watercourses, (II) evaluate innovative methods for biological communities (traditional and metabarcoding approaches), (III) identify the best analysis tool to assess the impact of aquaculture on receiving watercourses and (IV) assess efficacy of mitigation measures.

### Methods

In summer 2024, water quality of the receiving water courses was monitored for a group of 16 trout farms using freshwater macroinvertebrates (Indice Biotico Estesio, IBE) and diatom index (ICMI, Italian Water Framework Directive 2000/60/EC method). Diatom communities were further categorized into ecological guilds to provide insights into trophic conditions. To estimate nutrient enrichment, periphyton samples were collected for chlorophyll-a concentration and ash free dry mass analysis. Water samples were also collected at upstream and downstream stations to retrieve physicochemical parameters (e.g. O<sub>2</sub> saturation, COD, BOD<sub>5</sub>, total phosphorus, nitrate-N, ammonium-N, *Escherichia coli* concentration) and to calculate LIMeco (Italian WFD 2000/60/EC method) and LIM indices.

Farms monitored in this study are characterized by a flow-through scheme, which is widely applied in Trentino, due to rapid water renewal, optimal oxygen concentration and suspended solids removal, which allow optimal rearing conditions for fish. In some facilities, liquid oxygen is applied to support the production, and effluent treatment systems have been implemented to minimize environmental impacts.

Three trout farms that will implement drum filters within the project were selected to assess the mitigation potential. In addition to the previously described methods, quantitative analyses of the macroinvertebrate community were also performed. Five Surber net replicates were collected for one upstream and one downstream sampling station, and macroinvertebrates were identified to genus/family level. STAR-ICMi index, the official Italian method based on freshwater macroinvertebrates under WFD, was also calculated.

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Main compositional (e.g. 1-GOLD, ASPT, EPT, EPTD, taxa richness, Shannon index) and functional feeding group metrics (e.g. percentage of grazers, shredders, collectors, Shredders/Collectors ratio) were calculated to identify those most sensitive to trout farm impacts. Differences between upstream and downstream stations values were assessed using the Mann-Whitney U test.

### **Results and Discussion**

IBE results from the summer 2024 campaign indicate that most upstream stations were in ‘Quality Class I’, with limited changes observed at downstream stations (where the lowest class recorded is II). These findings agreed with the preliminary ICMI results, showing little or no impact on receiving watercourses. Diatom guild analysis further supported these conclusions, revealing the dominance of oligotrophic and low-profile taxa, which are typical of low-nutrient, and fast-flowing environments.

In all three farms that will apply impact-mitigation measures, %OC (percentage of oligochaetes and chironomids) showed a significant increase observed at downstream stations. In the two most impacted sites (based on IBE), this is accompanied by a decrease in 1-GOLD, ASPT, and percentage of grazers, along with an increase in the percentage of filter feeders. These trends suggest a community shift toward pollution-tolerant taxa feeding on fine particulate organic matter derived from aquaculture effluents, corroborating existing literature.

LIMEco assessments placed most stations in ‘Quality Class I’, with only three showing lower quality (the lowest being Class IV). LIM appeared to be more stringent, with nearly half of the stations classified as Class II and the rest as Class I. Among chemical parameters, a general increase in total phosphorus and total ammonium concentrations were observed at downstream stations, likely attributable to fish excreta and uneaten feed.

### **What’s next?**

For the three selected trout farms, biological and chemical analyses will be repeated under different seasonally conditions before-and-after the installation of drum filters. These data, along with a bulk eDNA metabarcoding approach, will be used to assess the effectiveness of the mitigation measures, understand how these methodologies can be integrated and develop new metrics and indices that are specifically suited to detecting trout farming-related impacts in montane lotic ecosystems.