ATTI CONVEGNO

APPLICATION OF DIGITAL DROPLET PCR FOR THE QUANTITATIVE ASSESSMENT AND RANKING OF INFESTATION SEVERITY IN RAINBOW TROUT NATURALLY AFFECTED BY PROLIFERATIVE KIDNEY DISEASE

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The RESILTROUT project (Resilient Aquaculture to Global Climate Change: Research Supporting Italian Rainbow Trout Production) aims to identify DNA markers for genomic selection to manage some of the main challenges for rainbow trout productions; among these the parasitic disease proliferative kidney disease (PKD) affecting salmonids across Europe and North America, with significant economic implications for both wild and farmed populations. *Tetracapsuloides bryosalmonae* (myxozoa) is the etiological agent of PKD. It is considered one of the most severe parasitic diseases in aquaculture, with seasonal outbreaks typically occurring between late spring and early autumn, coinciding with elevated water temperatures. During this period, invertebrate bryozoans release spores which infect fish via the gills or skin. Parasites then circulate preferentially to organs such as the kidney, spleen, and liver, where they proliferate. Rainbow trout is an irregular, dead-end host for *T. bryosalmonae* and only occasionally survives severe infections with mortality rates reaching up to 95% in hatchery-reared fish. Vaccines and therapies are not yet available to control PKD. At this purpose a genome-wide association study (GWAS) was carried out using a medium-density SNP commercial array on dead and surviving fish from natural outbreaks. Cases were defined through the presence of specific internal lesions, in particular kidney hyperplasia, spleen swelling and end-point PCR for *T. bryosalmonae* detection as described by Kent et al. (1998), amplifying a 435-bp segment of the SSU-rDNA gene. Considering PKD caused a general immunosuppression, a ranking of infestation was assessed to support the role of *T. bryosalmonae* as primary cause of mortality. A Digital droplet PCR (ddPCR) was, therefore, developed for absolute quantification and phenotype definition, using specific primers and probes described for real-time PCR by Sieber et al. (2023). Out of 990 total samples analyzed by endpoint PCR, 43% (n=426) tested positive for *T. bryosalmonae*. Among these, 368 samples were further analyzed by ddPCR, allowing quantification of pathogen load and classification into five infestation severity levels: very low (<10 copies/µL), low (10–99 copies/µL), moderate (100–999 copies/µL), high (1,000–9,999 copies/µL), and very high (>10,000 copies/µL). The majority of positive samples fell into the moderate, high and very high categories, representing 81% of the total. Nevertheless all the samples positive at end-point PCR even if with a low infestation level and in absence of major bacterial pathogens were included in the GWAS as cases. Moreover, a statistical model considering togheter (date of death, morphometric parameters, presence of other pathogens, internal lesions, end-point results, DDPCR results) has been tested. Phenotype definition is one of the bias affecting GWAS studies and the the unambiguous definition of the cases is substantial to avoid false associations.

Research funded by Italian Ministry of Agriculture, Food and Forestry, RESILTROUT-6CFP Supply Chain Program, Project Code 23E03