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Impact of abiotic factors and husbandry on saprolegniosis in salmonid farms

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ABSTRACT

Oomycetes of the genus *Saprolegnia* are widespread in freshwater environment and are among the main pathogens causing economic losses in salmonid aquaculture.

Infections by mycotic agents in fish farming are generally considered to result from chronic stress and poor fish condition associated with water quality problems, adverse environmental conditions, frequent/rough/incorrect handling, concurrent infections, physiological changes associated with reproduction and immunocompromised animals.

To identify risk factors for *Saprolegnia* infections in trout and Atlantic salmon farming, longitudinal studies were carried out in different Italian, Spanish, and Scottish fish farms. Prevalence of saprolegniosis and fish mortality were monitored over time and statistically analysed with respect to husbandry and environmental factors.

Overall, statistical results by production cycle (trout vs salmon farming) and by country indicate that the prevalence of *Saprolegnia* may be influenced by peculiarities of the culture system and farming environment. Nevertheless, a specific set of parameters, including lower water temperature, and handling procedures increased *Saprolegnia* prevalence in all the considered farms. Particularly, in trout farms *Saprolegnia* infections represented an important contribution to mortality, and prevalence was influenced by water temperature and pH, and by fish density within the tanks. Similarly, temperature and water quality were the main factors influencing the prevalence of *Saprolegnia* in Atlantic salmon farms. Moreover, molecular analyses confirmed the role of *S. parasitica* as the main pathogenic oomycete in trout and salmon farming in the considered countries. The identification of risk factors for introduction and increase of *Saprolegnia* infection in fish farms will allow the correct design of biosecurity and pathogen control strategies.

1. Introduction

Infections caused by oomycetes of the genus *Saprolegnia* represent a major problem in salmonid aquaculture (van den Berg et al., 2013). *Saprolegnia* spp. are considered ubiquitous in freshwater environments, including aquaculture facilities (Pavić et al., 2021; Tedesco et al., 2021), therefore both farmed and wild fish are continuously exposed to these potentially pathogenic organisms.

It is known that fish possess different defence mechanisms against oomycete agents, including a cellular immune response (Sohnle and Chusid, 1983) and a mucus layer that displays fungistatic activity and helps the shedding of attached spores (Willoughby, 1989). Nevertheless,

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several physiological, husbandry and environmental factors are believed to play a significant role in increasing susceptibility to *Saprolegnia* in farmed fish (Carballo et al., 1995; Richards and Pickering, 1978). In the natural environment, physiological changes in epidermal structure during sexual maturation associated with a decrease in mucous cells favour a successful establishment of *Saprolegnia* infection (Richards and Pickering, 1978).

Within fish farms, predisposing factors for saprolegniosis are more numerous than those in the natural environment: overcrowding with low oxygenation (Klinger and Francis-Floyd, 1996), insufficient flow rate (White, 1975), infrequent removal of dead fish and eggs can result in higher spore load in the farming unit (Bruno et al., 2011). Simultaneously, frequent or incorrect handling procedures resulting in damage of the fish skin and in increased stress have been associated to higher susceptibility of the fish to saprolegniosis (Beckmann et al., 2020; Bruno et al., 2011); an important contribution is also given by inadequate diet and nutritional deficiencies, such as insufficient supply of ascorbic acid, important for collagen production and for tissue regeneration (Ashley et al., 1975).

In addition, infection with *Saprolegnia* can be secondary to the presence of viral or bacterial pathogens (Egusa, 1965; O'Brien, 1974) and to the presence of ectoparasites able to cause mechanical damage to fish tissues (Johnsen, 1978).

Several abiotic factors can affect the distribution of oomycetes and the prevalence of saprolegniosis. As reported in the literature, optimal oxygen concentration for *Saprolegnia* spp. development is comprised between 8 and 12 mg/L (Voronin, 2008). Mycelial growth and zoospore production are favoured by acidic conditions (Kitancharoen et al., 1996; Peduzzi, 1991). With respect to temperature, different *Saprolegnia* species display distinct optimal growth temperature. *S. parasitica* is considered eurythermous, however in vitro tests showed increased mycelial growth and zoospore production at lower water temperatures (Kitancharoen et al., 1996). Further microbiological in vitro experiments showed that *S. parasitica* mycelial growth rate was highest between 15 and 25 °C, while zoospore viability and sporulation efficiency peaked at around 10 °C (Matthews, 2019). Thermal stress in fish resulting from increased water temperatures has also been associated to higher susceptibility to *S. parasitica* and higher disease severity (Casas-Mulet et al., 2021; Stewart et al., 2018). On the other hand, field studies carried out in fish ponds failed to detect *Saprolegnia* during warmer months, from April to October (Falk and Polacheck, 2016).

Water quality plays a major role in influencing the susceptibility of fish to saprolegniosis. The presence of high organic load and nitrogen compounds have been associated with increased *S. parasitica* infections (Pavić et al., 2022; Toor et al., 1983). Furthermore, the occurrence of toxic or harmful compounds could represent an important predisposing factor; trout exposed to sublethal levels of ammonia and nitrites are more susceptible to saprolegniosis (Carballo and Muñoz, 1991).

The amount of laboratory and field evidence collected so far and available in the literature would therefore suggest that a complex mix of abiotic and biotic factors affects the development of saprolegniosis. However, epidemiological longitudinal studies aimed at clarifying this aspect are rare (Abd El Aziz et al., 2004; Duan et al., 2018; Muir et al., 2015).

The present work is aimed at identifying risk factors for *Saprolegnia* infections in salmonid aquaculture, through longitudinal studies carried out in Italian and Spanish trout farms as well as Scottish Atlantic salmon farms. Prevalence of saprolegniosis and fish mortality were monitored over time and associated with environmental parameters, management practices, treatments and presence/absence of other pathogens.

2. Materials and methods

2.1. Trout farms

2.1.1. Data collection

Five trout farms in northern Italy and five trout farms in Spain (Fig. 1A, B) were selected based on the documented presence of different management risk factors, besides temperature, and other environmental factors, for saprolegniosis (e.g. vaccination, stripping, grading, frequent tank change). In the selected Italian farms, cultured trout species included rainbow trout, *Onchorhynchus mykiss* (RBT), marble trout, *Salmo marmoratus* (MT) and brown trout, *Salmo trutta* (BT); particularly, marble trout were mainly cultured for restocking, while brown trout and rainbow trout were farmed for food production. In Spanish farms, only *S. trutta* farmed for restocking were monitored. In both Italian and Spanish farms, fish were cultured in raceway systems with concrete tanks that receive water from nearby natural water bodies (river, resurgence).

Italian farms were monitored over a year and were visited on a monthly basis during the cold season (October–April), corresponding to higher likelihood for saprolegniosis, and once during summer (July) for a total of eight visits per farm. Spanish farms were monitored regularly (six visits/year, on average), with higher frequency during colder months.

During each visit, environmental parameters (pH, temperature and dissolved oxygen) were recorded and tanks were subjected to clinical inspection of fish (visual inspection of all the fish within each tank) to assess prevalence of lesions referable to saprolegniosis. Questionnaires were handled to fish farmers to obtain detailed information about management practices, fish handling, presence of concurrent infections, administration of treatments, and density in terms of total biomass and mortality.

2.1.2. Isolates culture

During the first visit, five fish with signs of saprolegniosis were sampled from each farm in order to identify the circulating species of *Saprolegnia*. Two tufts of mycelia from two different fish lesions were seeded on glucose-yeast extract agar (Min et al., 1994) with penicillin G (6 mg/l) and oxolinic acid (10 mg/l) (Alderman and Polglase, 1986) (GY + P + Ox). Isolates were incubated at 18 °C and re-cultured until pure cultures were obtained.

2.1.3. Isolates molecular identification

DNA extraction was performed using DNeasy Plant Mini Kit (QIAGEN, Valencia, California, USA). The internal transcribed spacer region (ITS) was amplified using universal primers for eukaryotes ITS5 and ITS4 (White et al., 1990), under the conditions described in Sandoval-Sierra et al. (2014). Amplified products were sequenced using an automated sequencer (Applied Biosystems 3730xl DNA, Macrogen, Netherlands). For each isolate, the consensus sequences for the ITS region were assembled and edited using the program Geneious v6.14 (Kearse et al., 2012).

Sequences were aligned using Mafft version 7.2 (Katoh and Standley, 2013) and the algorithm G-INS-I (Katoh and Toh, 2008a, 2008b). The sequences were analysed using Maximum Likelihood model, and the isolates were assigned to species based on reference sequences for molecular operational taxonomic units (MOTUs) described by Sandoval-Sierra et al. (2014). Maximum Likelihood analysis was carried out using the software RAxML v8.1 (Stamatakis, 2014). For this analysis, the random starting tree was selected. Clade support was assessed with 1000 bootstrap replicates after selecting the best tree from 100 trees generated.

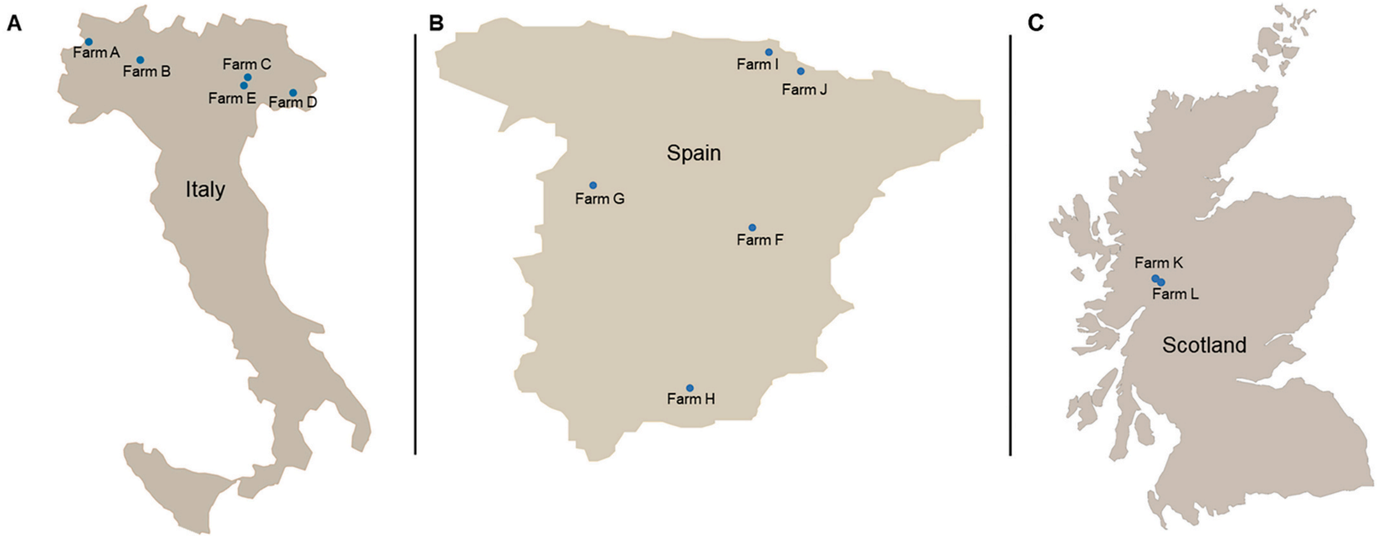

Fig. 1. Geographical localization of the trout and Atlantic salmon farms selected for the study. Cultured species: A = *S. trutta*; B, E = *O. mykiss*; C, D = *S. marmoratus*; F – J = *S. trutta*; K – L = *S. salar*.

2.2. Atlantic salmons farms

2.2.1. Data collection

Two Scottish farms with Atlantic salmon were monitored on a monthly basis for a year (Fig. 1C). In these farms, fish is cultured in an open-net pens system. The farms were within the same geographical area (connected lochs) and with the same husbandry procedures.

During each visit, environmental parameters (pH, temperature and dissolved oxygen) were recorded and tanks were subjected to clinical inspection of fish to assess prevalence of lesions referable to saprolegniosis. Questionnaires were handed to fish farmers in order to obtain detailed information about management practices, fish handling, presence of concurrent infections, administration of treatments, and density in terms of total biomass and mortality.

2.2.2. Isolates culture

Six-well and 90 mm plates with potato dextrose agar (PDA) supplemented with the antibiotics: vancomycin (100 mg/l) and ampicillin (500 mg/l) and the antifungal pimaricin (20 mg/l), PDAVAP, were prepared in advance and taken on site. Six fish were euthanized with a tricaine methanesulfonate (MS-222, Pharmaq) overdose followed by percussive stunning and the pectoral fins were cut off and plated as well as a sample of mycelia. Plates were sealed and transported from the site to the laboratory at ambient temperature, thereafter plates were incubated at 12 °C. Once individual samples showed clear mycelial growth an agar block was transferred to a new PDAVAP plate. This process was repeated until axenic cultures were obtained.

2.2.3. Isolates molecular identification

DNA was extracted following the protocol previously described by Zelaya-Molina et al., 2011). 50 μl of gDNA was cleaned from other nucleic acids by addition of 1 μl of RNAse A (20 mg/ml, Sigma Aldrich) and incubated for 30 min at 37 °C. The quality of the DNA was checked spectroscopically (NanoDrop) and visually on 1% (*w/v*) agarose gels.

Isolates were identified by PCR and subsequent sequencing reaction of the ITS region 1 and 2 including the 5.8 s rDNA region. For the ITS PCR and sequencing reaction the primers (ITS4 and ITS5) were used as described in White et al. (1990). The PCR reaction was performed in 25 μl reaction mix containing 5 μl of 5× colourless flexi buffer (Promega, UK), 10.75 μl nuclease free water, 5 mM $MgCl_2$, 0.2 mM dNTPs, 0.4 μM of each primers and 1.25 units Go*Taq G2* Polymerase (5u/μl, Promega) and 1 μl of DNA template (~50 ng/μl). The PCR reactions were run on a thermal cycler as follows: 1 cycle of initial denaturation (95 °C for 5 min), amplification for 30 cycles (95 °C for 30s, 57 °C for 1 min and 73 °C for 1 min 30s) and finally 1 cycle of final extension (73 °C for 7 min) followed by a 4 °C hold.

The ITS amplicons were sent to a commercial sequencing facility (Source Biosciences, Germany). The generated consensus sequences were compared with other oomycete ITS sequences from the GenBank sequence database using a BLASTN search algorithm. Using the software Molecular Evolutionary Genetics Analysis version 7 (MEGA7) (Kumar et al., 2016) a data set was compiled of ITS nucleotide sequences of *Saprolegnia* spp. and a sequence alignment was subsequently performed using the ClustalW algorithm. Finally, the evolutionary distances were computed using the maximum composite likelihood method with 1000 bootstraps.

2.3. Statistical analysis

Given that mortality and prevalence were expressed as the percentage of dead fish or fish with clinical signs of *Saprolegnia* infection, respectively, they were log-transformed prior to the calculation of Pearson's correlation coefficients between environmental and husbandry variables. The normality of the variables was assessed visually. Pearson's correlation coefficients were calculated for either all trout farms, trout farms with or without treatments, or all Atlantic salmon farms.

A principal component analysis (PCA) was then carried out to explore variability within the dataset and identify variables influencing prevalence and mortality in trout farms and Atlantic salmon farms. The component loadings were summarised for each analysis. Finally, logistic regressions were used to identify the factors impacting the presence of saprolegniosis in all farms across countries, and by country, using environmental and husbandry variables (temperature, pH, saturation, dissolved oxygen, density and stress level). The presence of saprolegniosis in the farm at the time of the sampling was expressed as a binary variable (0: absent, 1: present). Generalised linear models with a logit function were similarly fitted to the prevalence and the mortality transformed into the ratio of fish with *Saprolegnia* clinical signs or dead, respectively.

All statistical analyses were performed using the statistical software R 4.1.2 (R Core Team, 2021) with the packages *corrplot* (Wei and Simko, 2021), *ggplot2* (Wickham, 2016), *ggbiplot* (Vu, 2011) and *lme4* (Bates et al., 2015).

3. Results

3.1. Trout farms

All isolates from lesions obtained from farmed trout in the Italian/ Spanish farms selected for the study belonged to the species *Saprolegnia parasitica*.

3.1.1. Dataset description

In Italian trout farms (Supplementary material 1), the temperature varied between 3.4 and 22.7 (mean 10.2, standard deviation (sd) 4.1), pH values ranged between 6.12 and 8.28 (mean 7.69, sd 0.48), while dissolved oxygen levels were between 8.3 and 14.9 ppm (mean 11, sd 1.5) with a saturation of 66–155% (mean 97.8, sd 13). The density of fish in the tank was very variable among farms and across the observation period, ranging from 0.5 to 46.9 kg/m^3 (mean 12.9, sd 10). Similarly, the range of values for the prevalence of fish with *Saprolegnia* lesions (comprised between 0 and > 50%) and for the mortality (comprised between 0 and > 90%) was wide.

In Spanish trout farms (Supplementary material 1), the temperature varied between 3.6 and 26.4 (mean 11.1, sd 4.1), pH values ranged between 6.49 and 8.76 (mean 7.78, sd 0.63), while dissolved oxygen levels were between 7.5 and 9 ppm (mean 8.3, sd 0.4) with a saturation of 71.2–84.8% (mean 79.1, sd 3.9). The density of fish in the tank ranged between 5 and 7.5 kg/m^3. Prevalence varied between 0.1% and 10%, while mortality varied between 1% and 7%.

In both Italian and Spanish trout farms, potentially stressful handling procedures considered for the study included tank change, stripping of broodstock, operations related to grading and intraperitoneal vaccination. The frequency of such handling procedures was variable among farms: three Spanish farms did not perform any manipulation, while other trout farms performed different types of manipulations with frequency varying from around 15 to 50% of observations (Fig. 2).

With respect to treatment administration, two Italian farms did not report any treatment during the monitoring year (Fig. 3). Administered treatments were farm specific, with hydrogen peroxide and formalin being used exclusively in Spanish farms, two Italian farms administering copper sulphate and peroxygenate sanitizers during colder months, and other Italian farms administering medicated feed containing flumequin and formulations containing other antibiotics for the treatment of bacterial diseases.

Prevalence and mortality varied substantially between farms (Supplementary material 2). Environmental and husbandry variables also varied considerably between farms (Fig. 4). Pearson's correlation coefficients calculated using data from all trout farms combined showed that prevalence was strongly positively associated with mortality ($\rho = 0.54$), and weakly negatively associated with pH ($\rho = -0.24$) and temperature ($\rho = -0.30$) (Fig. 5A). In samples associated with treatments, prevalence was negatively associated with dissolved oxygen ($\rho = -0.46$) and pH ($\rho = -0.23$), but positively with density ($\rho = 0.31$) and mortality ($\rho = 0.28$) (Fig. 5B). In samples associated with no treatments, prevalence was negatively associated with temperature ($\rho = -0.48$), density ($\rho = -0.33$) and pH ($\rho = -0.22$), but strongly positively associated with mortality ($\rho = 0.67$) (Fig. 5C).

The PCA applied to all trout farms confirmed substantial variability between farms (Fig. 6, Table 1). Density, prevalence and temperature had the strongest influence on the first component, capturing 30% of the variance, while mortality, prevalence and dissolved oxygen had the strongest influence on the second component, captured 26% of the variance. Mortality and prevalence had both a strong positive relationship with the second component, while pH and DO had both a negative relationship with the second component. Differences between farms seem to be strongly influenced by differences in density.

3.2. Atlantic salmon farms

For the Atlantic salmon farms, most isolates obtained belonged to *Saprolegnia parasitica* species, with a few other *Saprolegnia* species isolated, such as *S. australis, S. diclina, S. parahypogyna*, and *S. delica*.

3.2.1. Dataset description

In Scottish farms (Supplementary material 1), the water temperature varied between 6.1 and 16.7 (mean 10.7 ± 3.6), pH values ranged between 4.5 and 8 (mean 6.78 ± 0.83), while dissolved oxygen levels were

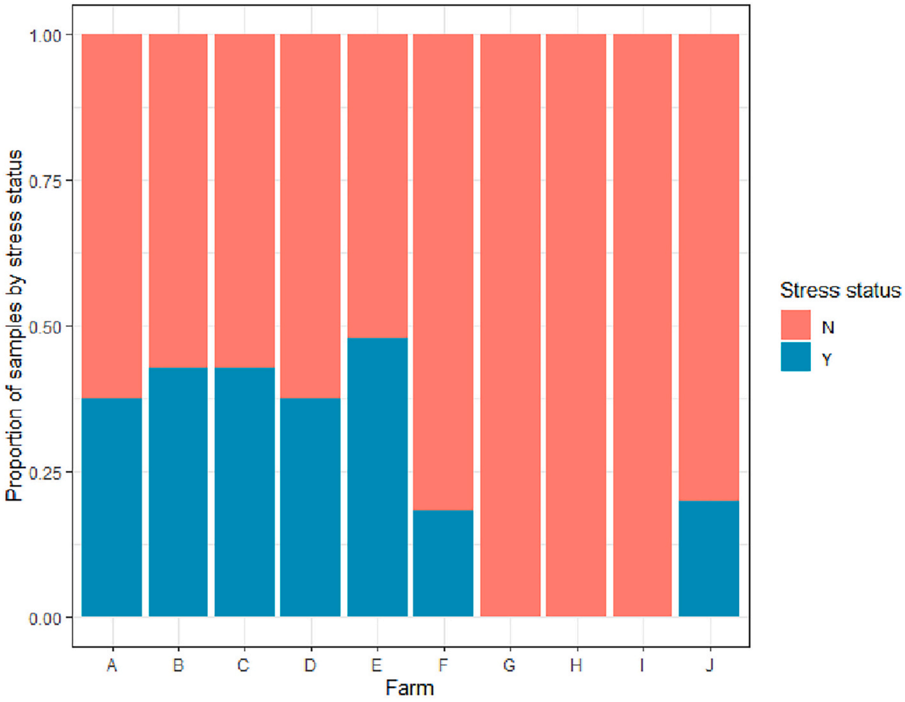

Fig. 2. Trout farms: frequency of occurrence of handling procedures (stress).

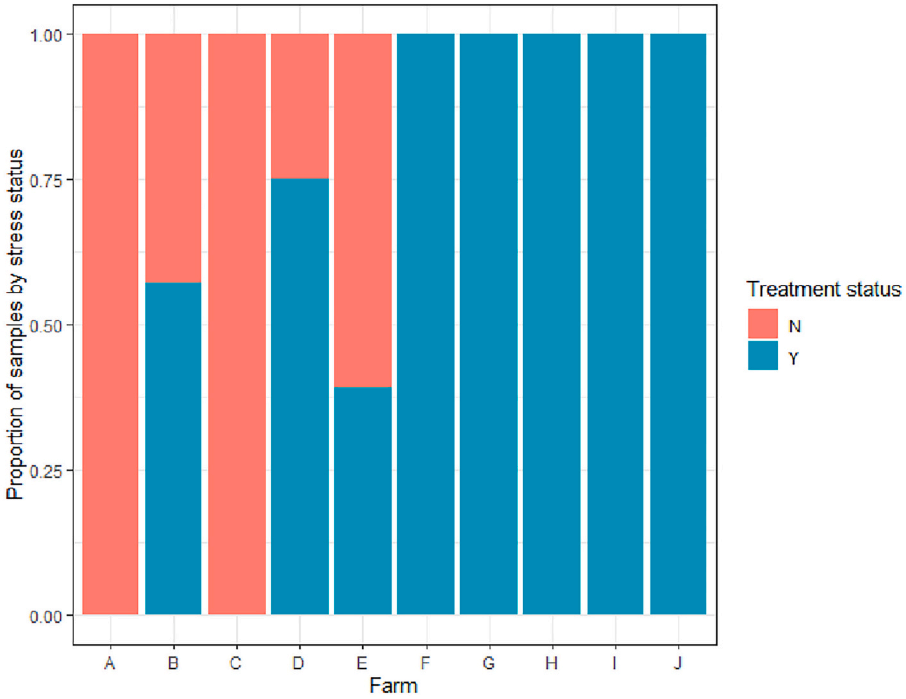

Fig. 3. Trout farms: frequency of treatment administration.

between 7.9 and 13.5 ppm (mean 10.7 ± 1.3) with a saturation of 90–94% (mean 91.7 ± 1.1). Scottish loch temperatures tend to be warmer in winter and cooler in summer than adjacent rivers. Temperatures show greatest fluctuations and between-site variability in the summer months (Supplementary material 3).

Between the two Atlantic salmon farms no statistical significance was found regarding temperature, pH and dissolved oxygen (Fig. 7).

The density of fish varied from 30 to 60 kg/m^3 in the two farms. Regarding prevalence values, one farm had constant saprolegniosis issues, with prevalence ranging from <8% to 20%, while the other farm rarely had saprolegniosis (10% only during one observation). Potentially stressful manipulations in Atlantic salmon farms are of different types (vaccination, crowding, grading, introduction of new fish) with frequency varying from around 15 to 50% of observations.

The frequency of treatments was variable between the two Atlantic salmon farms: the farm with minimal saprolegniosis problems only performs a few treatments per year, mostly for prophylaxis purposes, while the second farm treats on a regular basis due to the high incidence of saprolegniosis. Nevertheless, both farms use the same treatment (formalin and bronopol) and regime.

Pearson's correlation coefficient showed a strong positive association between prevalence and density ($\rho = 0.86$, Fig. 9). All the other correlations were weak. The PCA showed that prevalence and density had both a strongly negative relationship with the first component (Fig. 8, Table 2). Both variables had the strongest influence on the first component, capturing 40% of the variance. The temperature and dissolved oxygen having the strongest influence on the second component captured 27% of the variance. Both farms were distinguished by the first component.

Nevertheless, a positive Pearson's correlation was found between temperature and pH for farm K ($\rho = 0.50$) and farm L ($\rho = 0.25$) and in general ($\rho = 0.08$). Prevalence was positively associated with the density ($\rho = 0.86$) (Fig. 9).

3.3. *Full dataset analysis*

Generalised linear models with logit link function were fitted to data from all farms and showed no effect of environmental and husbandry variables on prevalence (clinical signs of *Saprolegnia* infection) and mortality (dead fish) (Table 3). The logistic model fitted to *Saprolegnia* presence (binary response) across all farms, however, indicated that *Saprolegnia* was more likely to be detected with the increase in stress and decrease in temperature at the 5% significance level ($\beta = 3.54$, $z = 4.68$, $p = 2.92e10^{-6}$; $\beta = -0.22$, $z = -2.35$, $p = 0.02$; respectively, $R^2 = 0.40$).

Given disparities in variables between farms and country datasets, logistic and generalised linear models were applied to farms per country. No effect of environmental and husbandry variables on prevalence and mortality was found in Italian farms, but *Saprolegnia* presence was more likely under stressful conditions and decrease in temperature ($\beta = 3.32$, $z = 2.64$, $p = 0.01$; $\beta = -1.11$, $z = -2.02$, $p = 0.04$; respectively, $R^2 = 0.56$). Due to the limited sample size for Spanish and Scottish farms, models could not be applied to *Saprolegnia* presence and showed no effect of environmental and husbandry variables on prevalence and mortality.

4. Discussion

The crowding, sorting and regrouping of fish as they grow is a common practice in fish farming, as the distribution of fish sizes can become variable over time. According to the results of our study, these manipulations are most likely to produce an increase in prevalence of saprolegniosis, even in the presence of environmental factors (i.e. higher water temperature, lower density) that would be less favourable to *Saprolegnia parasitica* development.

Concerning the trout farms under study, despite the possible occurrence of other sources of mortality, results of linear regression analysis highlight that saprolegniosis represents a major contribution to mortality. This observation is also confirmed by the positive correlation between prevalence and mortality, as emerged from the analysis of Pearson's correlation coefficients, in the presence and in absence of treatments/manipulations.

With respect to temperature, its negative correlation with prevalence could be attenuated by the administration of chemical treatments which, as reported by farmers, was more frequent during the colder months.

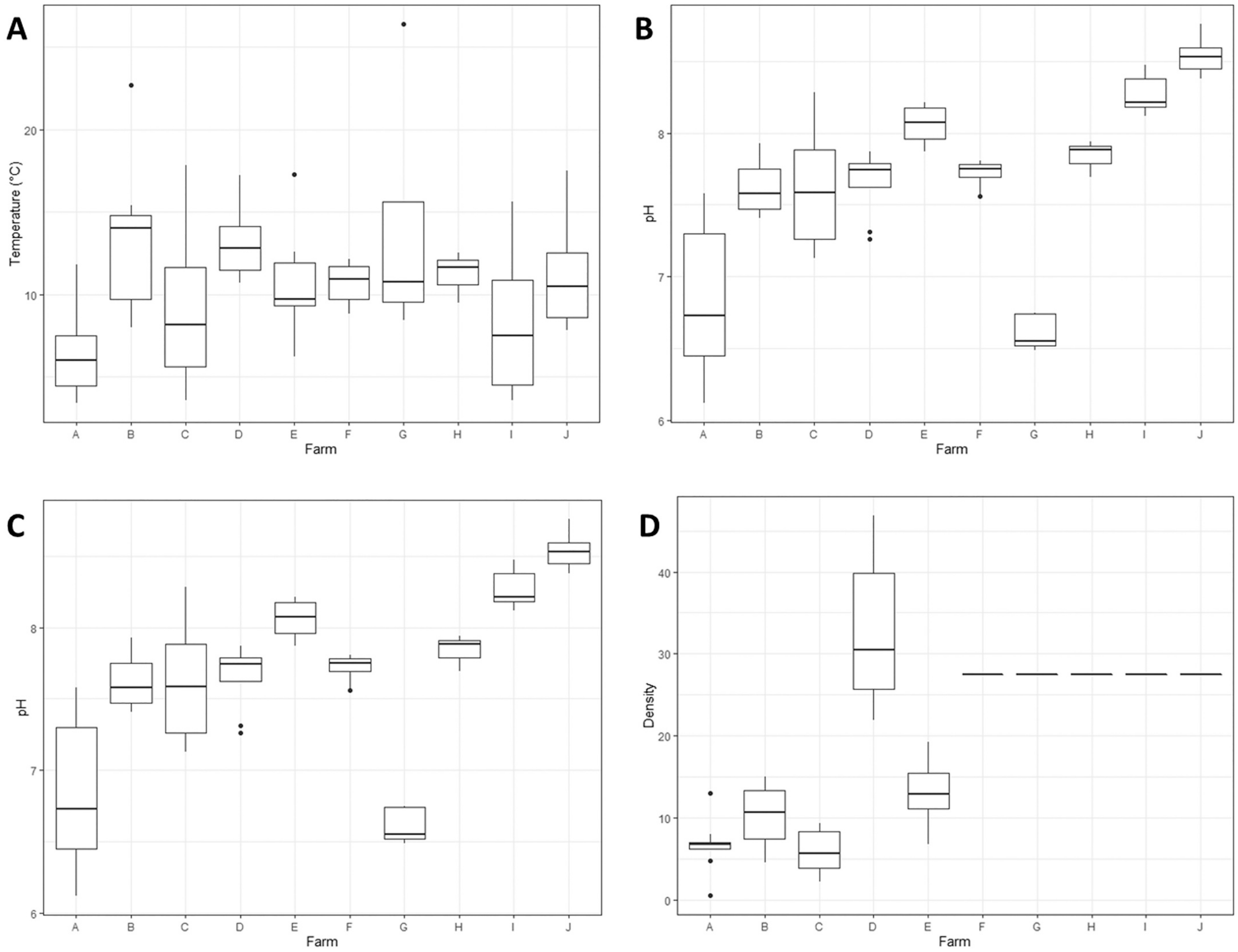

Fig. 4. Variability among trout farms: environmental parameters and biomass.

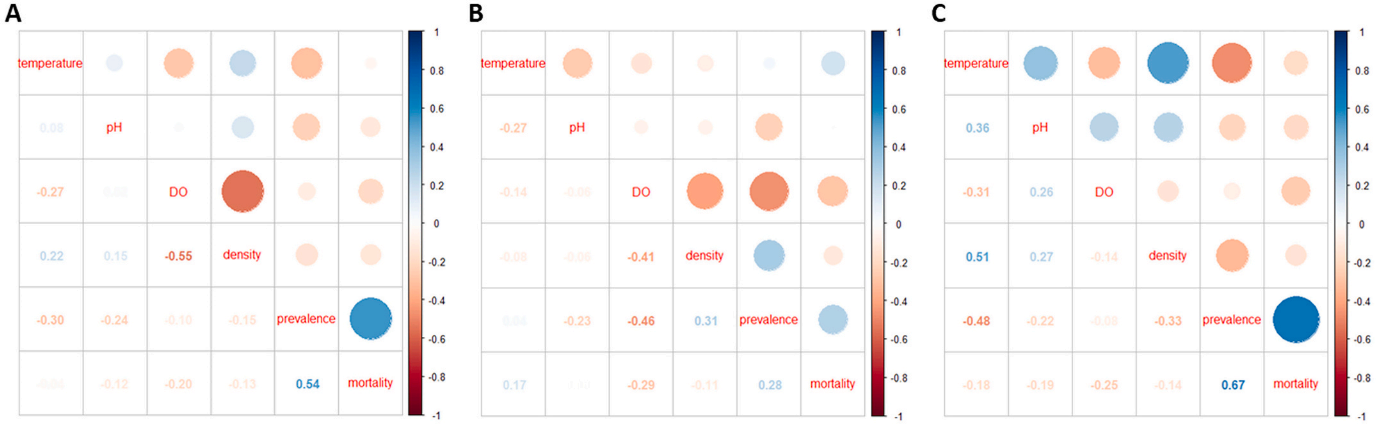

Fig. 5. Pearson's correlation coefficients: in all trout farms (A); in samples associated with treatment(B); in samples associated with no treatments, (C).

This hypothesis is supported by the observation that, by excluding the contribution of treatments from the analysis, the negative correlation between these two variables was strengthened. The occurrence of an inverse relationship between prevalence of saprolegniosis and temperature is also confirmed by linear regression analysis and is coherent with previous reports based on laboratory trials and field observations; particularly, in channel catfish susceptibility to saprolegniosis is reported to increase during colder periods (Bly et al., 1993), therefore *Saprolegnia* infection in catfish is often referred to as "winter kill". A study performed on strains of *S. parasitica* isolated from brown trout during a severe mortality outbreak in Spanish farms reported higher percentage of motile zoospores and radial growth at lower water

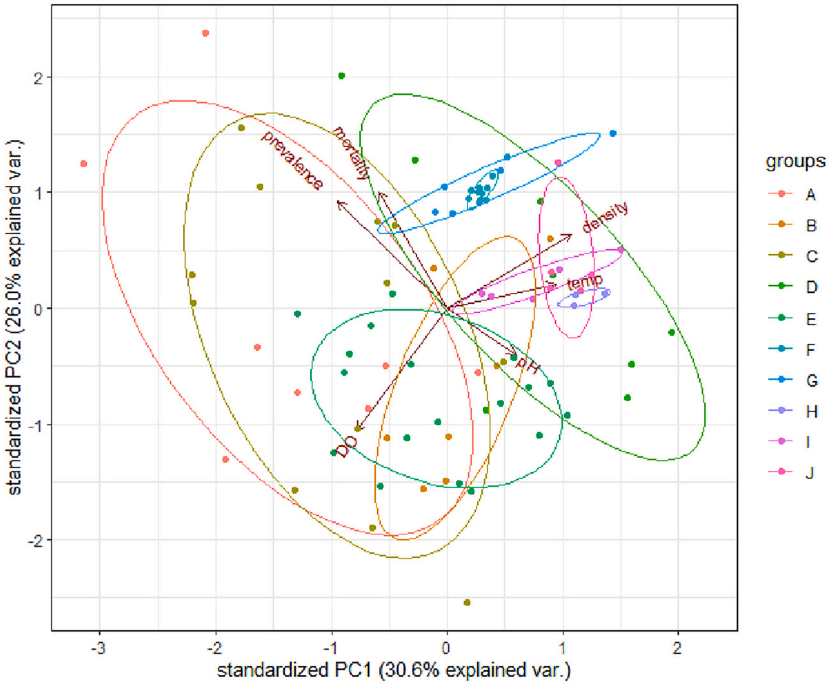

Fig. 6. Trout farms: Principal component analysis (PCA).

Table 1
Loadings from the first 2 principal components of the PCA for trout farms.

Variable	Component 1 (30.6%)	Component 2 (26%)
temperature	0.45773	0.1065
pH	0.28997	−0.2131
DO	−0.3818	−0.5585
density	0.51839	0.33704
prevalence	−0.459	0.48889
mortality	−0.2851	0.52797

temperatures (Diéguez-Uribeondo et al., 1996). Other in vitro experiments showed that lower temperature (10 °C) and acidic conditions (pH 4.8) tend to favour both mycelial growth and motile zoospores production in *S. parasitica* and *S. diclina* strains (Kitancharoen et al., 1996); acidic conditions are generally considered as most suitable for hyphal growth (pH 5.0–5.5) and zoospore production (pH 6.2) (Peduzzi, 1991).

Despite the correlation between saprolegniosis prevalence and mortality, as previously mentioned, the lack of significant correlation between mortality with temperature and pH resulting from trout farms may be related to the possibility that other mortality causes other than saprolegniosis are involved. During the Spanish/Italian longitudinal study, particularly in warmer months, farmers reported the occurrence of either confirmed or suspected cases of bacterial infections, such as enteric red mouth disease, furunculosis, lactococcosis and streptococcosis. Moreover, the administration of treatments for saprolegniosis during colder months may have contributed to reduce the mortality related to *Saprolegnia*.

Another interesting aspect concerns the fish density within the faming unit. Information available in the literature suggest that mechanical damage resulting from high stocking density of farmed trout may lead to increased incidence of saprolegniosis (Richards and Pickering, 1978). On the contrary, in the trout farms considered, our results indicate a negative (although weak) association between prevalence and density, which may be related to the occurrence of different management practices on the monitored tanks. In fact, common handling practices in fish farming involve sorting and moving fish to larger units as they grow. This could result in increased prevalence of *Saprolegnia* due to fish stress and possible lesions produced during handling (Beckmann et al., 2020). In one of the Italian farms considered for our study, the monitored fish were also subjected to intraperitoneal vaccination. After this procedure, the fish were moved to larger units, therefore at the time of recording, the population density was decreased, however prevalence of saprolegniosis had increased from 0 to 2–3%, possibly due

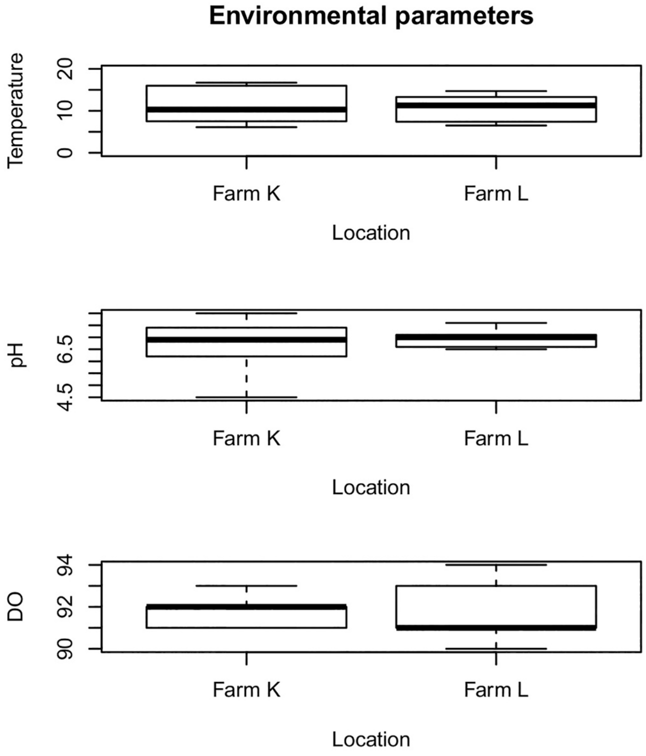

Fig. 7. Boxplot of different environmental parameters within the different sampling sites used in the Atlantic salmon study. No statistical significance was found between the sites.

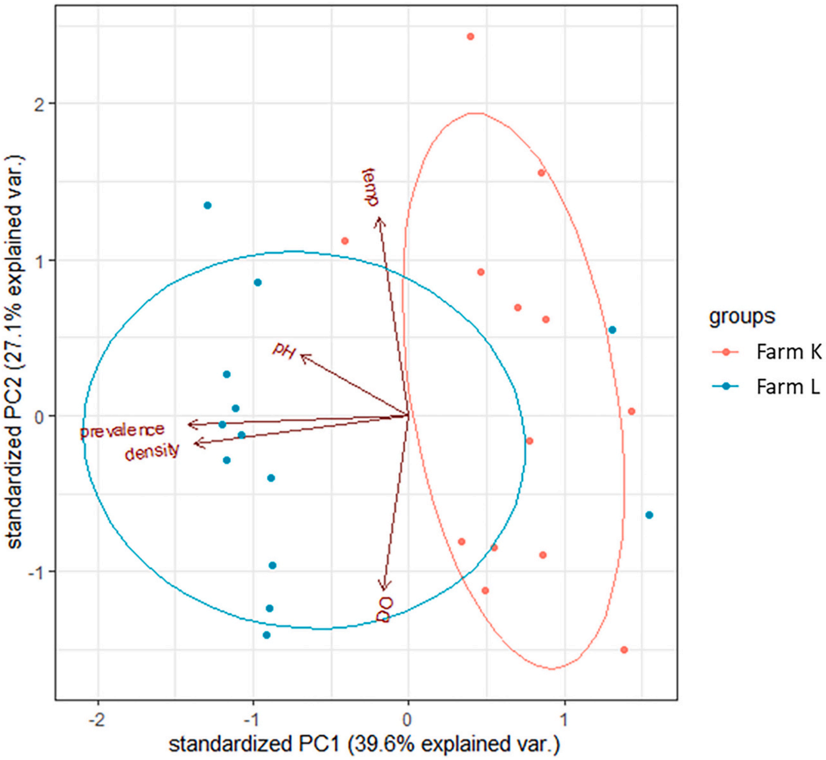

Fig. 8. Principal component analysis showing the variables influence.

Table 2
Loadings from the first 2 principal components of the PCA for Atlantic salmon farms.

Variable	Component 1 (39.6%)	Component 2 (27.1%)
temperature	−0.0896	0.7264
pH	−0.3302	0.2208
DO	−0.076	−0.6419
density	−0.6522	−0.1018
prevalence	−0.6722	−0.0340

to manipulation and despite the higher water temperature recorded. Therefore, the negative association between prevalence and density highlighted in trout farms in this study, should be considered as strongly influenced by management practices within each farm.

During the Scottish longitudinal study, particularly in warmer months, farmers reported the occurrence of either confirmed or suspected cases of other infections, such as furunculosis. Also, the prophylaxis treatments regime for saprolegniosis during colder months may have contributed to reduce *Saprolegnia* incidence.

Interestingly, the higher the fish density within a farm the higher is the prevalence of the disease as seen by the Pearson correlation analysis. This fact comes in accordance with the data from Richards and Pickering (1978). As per the trout farms, in the Atlantic salmon farms handling procedures play a role, especially vaccination and crowding;, these procedures have a high probability of causing lesions by physical abrasion on the fish resulting in a higher infection level. Although nowadays automated vaccination is getting to a standard procedure level, many Atlantic salmon farms, like the tested ones, still vaccinate manually increasing the chances of physical damage to the fish.

The loch temperature takes longer to respond to seasonal changes and rainfall events, due to larger water volume. Lochs also possess slightly acidic waters, due to underlying geology. It is believed, as described above, that temperature below 10 °C and acidic conditions tend to favour both mycelial growth and motile zoospores production in

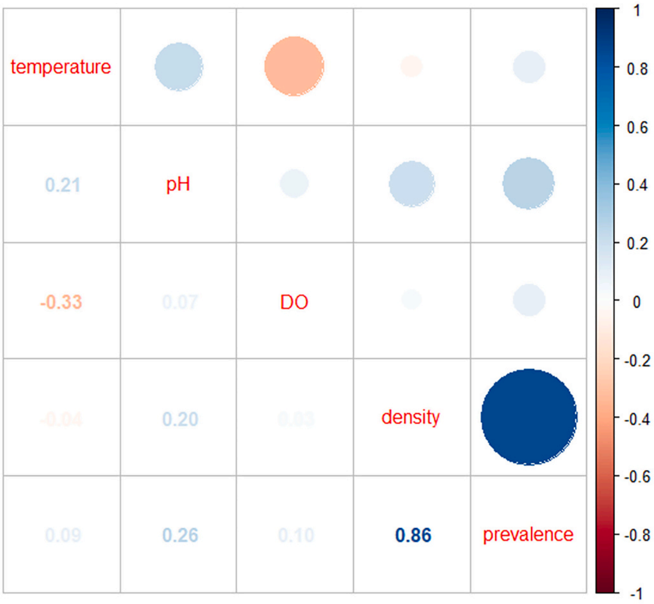

Fig. 9. Pearson correlation of the different parameters analysed. Note that the statistically significant values are plotted.

S. parasitica and *S. diclina* strains (Kitancharoen et al., 1996). Nevertheless, our results show a positive correlation between temperature and pH with saprolegniosis occurrence. We can only speculate the reason behind these results: a) the winter prophylaxis treatments masked the results; b) other important abiotic factors, besides temperature and pH, play a crucial role on saprolegniosis onset; c) a synergistic effect with other microorganisms or abiotic factor might take place. Further holistic and long duration studies are necessary to decipher the key risk factors.

Overall, results of statistical modelling by production cycle (trout vs

Table 3

Coefficients (β), z-values (z) and *p*-values (p) of logistic models fitted to mortality, prevalence and presence of *Saprolegnia* with associated R^2.

Variable		*temperature*	*pH*	*saturation*	*DO*	*density*	*stress*
mortality	β	0.025	−0.513	0.027	−0.038	0.027	1.835
$R^2 = 0.33$	z	0.105	−0.395	0.234	−0.039	0.487	1.350
	p	0.916	0.693	0.815	0.969	0.626	0.177
prevalence	β	−0.051	−0.320	−0.072	0.239	−0.001	1.393
$R^2 = 0.36$	z	−0.349	−0.547	−0.932	0.478	−0.028	1.556
	p	0.727	0.585	0.351	0.633	0.978	0.120
presence	β	−0.218	0.312	−0.020	0.597	0.03	3.537
$R^2 = 0.40$	z	−2.346	0.870	−0.502	2.004	1.718	4.676
	p	0.019	0.384	0.616	0.050	0.089	$2.92e10^{-6}$

salmon farming) and by country (Italy, Spain and Scotland) indicate that the prevalence of *Saprolegnia* could be influenced by different factors in relation to the farming practices, and to peculiarities of the culture system and of the farming environment. Particularly, results of the logistic model applied by country showed that temperature and handling have a statistically significant effect on the presence of *Saprolegnia* in Italy; however, the model did not highlight an effect of these variables analysed in Spain and Scotland indicating that the global effect of temperature and stress across all locations is mainly driven by the Italian dataset.

Production management practices in farms, involving the manipulation and adjustment of many variables daily, may pose challenges in carrying out complete longitudinal studies on selected tanks. Nevertheless, our work highlights a different influence of environmental and management factors on the presence and prevalence of *Saprolegnia*, and on mortality, which are possibly linked to peculiarities of the culture system and of the farming environment.

5. Conclusion

In every fish farm there are ultimately three essential elements present that determine health or disease of the fish. These are 1) the fish, 2) the pathogen and 3) the environment. Each element brings several parameters or risk factors that can, in most cases, be measured individually.

Over the last few years the incidence of saprolegniosis outbreaks in farms has increased significantly. Indeed, some sites have experienced high losses (10–15% or more) due to saprolegniosis, whereas some farms have stayed largely disease free. The reasons for some farms being affected more than others are, at present, not fully understood. Nevertheless, our work highlighted the role of a set of environmental parameters (i.e. temperature, pH and dissolved oxygen) and handling procedures in influencing saprolegniosis development. As emerged from our analyses, the influence of these parameters can be highly variable in relation to the culture system and to geographical/environmental factors; therefore, these factors should be considered following a case by case approach for a correct management of this parasitic infection. Despite this variability, when taking all farms into consideration, our results point towards water temperature and handling-related stress as the main factors involved in increasing *Saprolegnia* prevalence. More holistic studies will allow assessment of the full extension of the parameters involved and development of more tailored mitigation strategies.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Research data are shared as supplementary material

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2022.738679.

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