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Capture of *Saprolegnia parasitica* Spores in Flow-Through Aquaculture: First Observations

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ABSTRACT

Saprolegniosis, typically induced by oomycete *Saprolegnia parasitica*, is one of the most difficult pathogens in fish and other aquatic animals in freshwater systems. It is especially harmful for the endangered species landlocked salmon (*Salmo salar* m. *sebago*). Currently, there are only few alternatives to prevent and treat saprolegniosis occurrences, which can lead to major fish deaths and financial losses at fish farms. In this study, surface-modified cellulose materials were used at an experimental flow-through fish farm rearing landlocked salmon, which often suffers from saprolegniosis occurrences. The results showed that the material's cationic surfaces were able to capture the spores of *S. parasitica* (experimental part I and part II). The cellulose material was chemically modified with a high density of cationic quaternary ammonium groups, which performed better than a material with a weak cationic charge by amino groups obtained via physisorption of chitosan on the surface, resulting in fewer *S. parasitica* spores in the rearing tank water (experimental part I). The results are promising and offer a novel method for controlling saprolegniosis occurrences without harmful chemicals. However, certain environmental conditions (in experimental part II) inhibited the detection method (real-time quantitative polymerase chain reaction) used for the detection of *S. parasitica*. This highlights the need for further method development for the detection of *S. parasitica*. Overall, the results are promising in terms of reducing *S. parasitica* spores in rearing water and further controlling saprolegniosis occurrences. More process optimization is required to achieve the method's full potential in industrial scale processes.

1 | Introduction

Saprolegnia species, also referred to as water mould, belongs to fungal-like oomycetes, which are related to brown algae and diatoms (Baldauf et al. 2000; Erdei et al. 2023). The fish disease saprolegniosis is caused by *Saprolegnia* species, including *Saprolegnia parasitica*. It is a serious disease in the aquaculture industry and causes massive fish mortality in freshwater systems (Nam et al. 2022). It poses a serious threat to many species, including rainbow trout, salmon, tilapia, and carps in their different life stages (Shah et al. 2021), including the already endangered landlocked salmon *Salmo salar* m. *sebago*. In Finland, landlocked salmon lives in lake Vuoksi, lake Laatokka, and lake Vääninen, but at present, it is extremely endangered, and its existence mostly relies on the stocking and farming of the species. Furthermore, saprolegniosis poses threat to the production of roe, juvenile, and later life stages of fish both in flow-through (Y. Lankinen personal communication 2025) and in recirculating aquaculture systems in Scotland (Davis 2025).

Saprolegnia parasitica belongs to the family Saprolegniaceae, which is ubiquitous in freshwater ecosystems (Sakaguchi et al. 2019). Saprolegniosis infection can be visually observed via cottony grey or white growth on skin or gills of fish (Yanong 2003). In

aquaculture, the cause of the saprolegniosis outbreaks is not fully known, but it seems to be linked with inadequate water quality, mechanical injuries in fish, environmental stressors, and abrupt changes in rearing conditions (Ali et al. 2020; Lindholm-Lehto and Pylkkö 2024).

Saprolegnia parasitica multiplies on fish by branching hyphae and spreads into water via zoospores (Roberts 2012). The infection often starts on the head or fins and expands through branching mycelium in circles or curvilinear patterns throughout the body of fish (Engblom et al. 2023). Yolk sac fry and egg can also be infected (Roberts 2012). The infection can kill cells and destroy the skin of fish. The infection spreads into muscle tissue and blood vessels, causing advanced infection. The infected fish die from haemodilution due to skin loss and osmotic collapse. Unfortunately, there is no effective cure for the advanced disease.

In previous decades, malachite green was the most effective treatment for saprolegniosis, but it was banned in edible fish (in the European Union in 2000) due to its carcinogenic and toxic effects (Srivastava et al. 2004). Malachite green accumulates in fish flesh; it is difficult to remove and is harmful for fish farmers to use.

Since then, a variety of treatments, including formalin, hydrogen peroxide, sodium chloride, bronopol, formalin (Barnes et al. 2003; Branson 2002), peracetic acid (Jaafar et al. 2013), and potassium permanganate ($KMnO_4$; Zahran and Risha 2013), have been studied and used to control saprolegniosis (Ali et al. 2019; Lindholm-Lehto and Pylkkö 2024). However, these chemicals may be harmful for fish farm personnel, have adverse effects on aquatic environment, or accumulate in fish products (Thanikachalam et al. 2010; Özçelik et al. 2020). Based on European Union's biocide legislation (EU 2024/2421), a ban on the use of formalin and hydrogen peroxide to prevent saprolegniosis has recently been suggested in Finland. Prohibiting the use of formalin would be detrimental especially for roe and juvenile production, which highly relies on formalin's effect for preventing saprolegniosis. An alternative treatment agent is currently not available, and a ban on the use of formalin would end the commercial fish production in many cases.

Suitable compounds against saprolegniosis outbreaks have been searched among plant extracts with fungicidal properties, including *Nigella sativa*, *Punica granatum*, and *Thymus vulgaris*, to name a few (Mostafa et al. 2020; reviewed in Lindholm-Lehto and Pylkkö 2024). For example, essential oils (*Eucalyptus globules* and *Thymus daenensis*) were effective in suppressing fungal growth of *S. parasitica* (Salehi et al. 2015). Overall, currently accepted compounds and treatments against *Saprolegnia* spp. are scarce or inefficient, making the treatment of saprolegniosis problematic. Additionally, vaccination against saprolegniosis has been proposed, but its application in aquaculture is costly and impractical (Fregeneda-Grandes and Olesen 2007; Earle and Hintz 2014; Minor et al. 2014; Elgendy et al. 2023).

Cellulose is a raw material utilized in a variety of applications due to its abundancy, renewability, and versatile materials properties (Sjöström 1993). With the abundance of hydroxyl groups naturally in its structure, it possesses inherent hygroscopicity that induces the formation of hydrated layer on its surface, hindering the attachment of proteins and other foulants on the surface (Klemm 1998). The hydrated layer acts as a barrier towards fouling, and it can be further enhanced by adding ionic groups on the surface (Ding et al. 2019; Arandia et al. 2023). For the functionalization of the cellulose surface, the hydroxyl groups allow chemical modification with various functional groups. Alternatively, the inherent affinity of dissolved polysaccharides, such as functionalized cellulose derivatives or chitosan, towards cellulose can be used to modify cellulose surface via spontaneous polymer physisorption (Orelma et al. 2011).

Specific surface functionalization of cellulose material can be used for the selective binding of targeted compounds from aqueous environments. An attractive electrostatic interaction between the capturing material and the target species can be exploited in the capturing process. In such a context, various nanocellulose grades have been used for the capture of heavy metals (Ma et al. 2011; Isobe et al. 2013; Hokkanen et al. 2016) or organic dyes (Mohammed et al. 2015) from water. In addition to the electrostatic interaction, the morphology of the functional material is likely to play a role in capturing applications. The availability of cellulosic components in a variety of sizes and shapes, ranging from native nano to macroscale fibres or molecularly dissolved and regenerated structures, enables producing cellulosic constructs of various morphologies and material tunability in filtering and capturing applications.

In this study, we used the expected electrostatic attraction between cellulose fibres functionalized with cationic amine- or ammonium-based chemical groups and the inherent anionic surface charge (Rezinciuc et al. 2018; Wang et al. 2025) of *S. parasitica* spores. We hypothesized that this interaction enabled spontaneous enrichment and capturing of the spores simply by exposing the cationized fibres to the *S. parasitica* spores-containing water. In addition, the potential influence of the morphology of the cellulosic material for the mechanical attachment of spores was evaluated by comparing two different cellulosic fabrics as a capturing material: a densely bound woven cotton and a more loosely bound viscose non-woven.

The aim of this study was to develop and realize an environmentally friendly solution for decreasing free *S. parasitica* spores in aquaculture farm water by capturing the spores by cationized cellulose-based material. The study involves the preparation and characterization of the capturing material as well as introducing its applicability at the fish farm rearing landlocked salmon *S. Salar* m. *sebago*. Furthermore, the aim was to prevent lethal saprolegniosis occurrences in fish, especially for critically endangered landlocked salmon populations.

2 | Materials and Methods

2.1 | Modification of Cellulosic Fibers

The cellulosic fibre web was modified via two alternatives to cationize the materials' surfaces: (i) covalent modification of hydroxyl groups of cellulose with glycidyl trimethyl ammonium chloride (GTAC) and (ii) non-covalent modification of cellulose via the physisorption of chitosan (Orelma et al. 2011). The

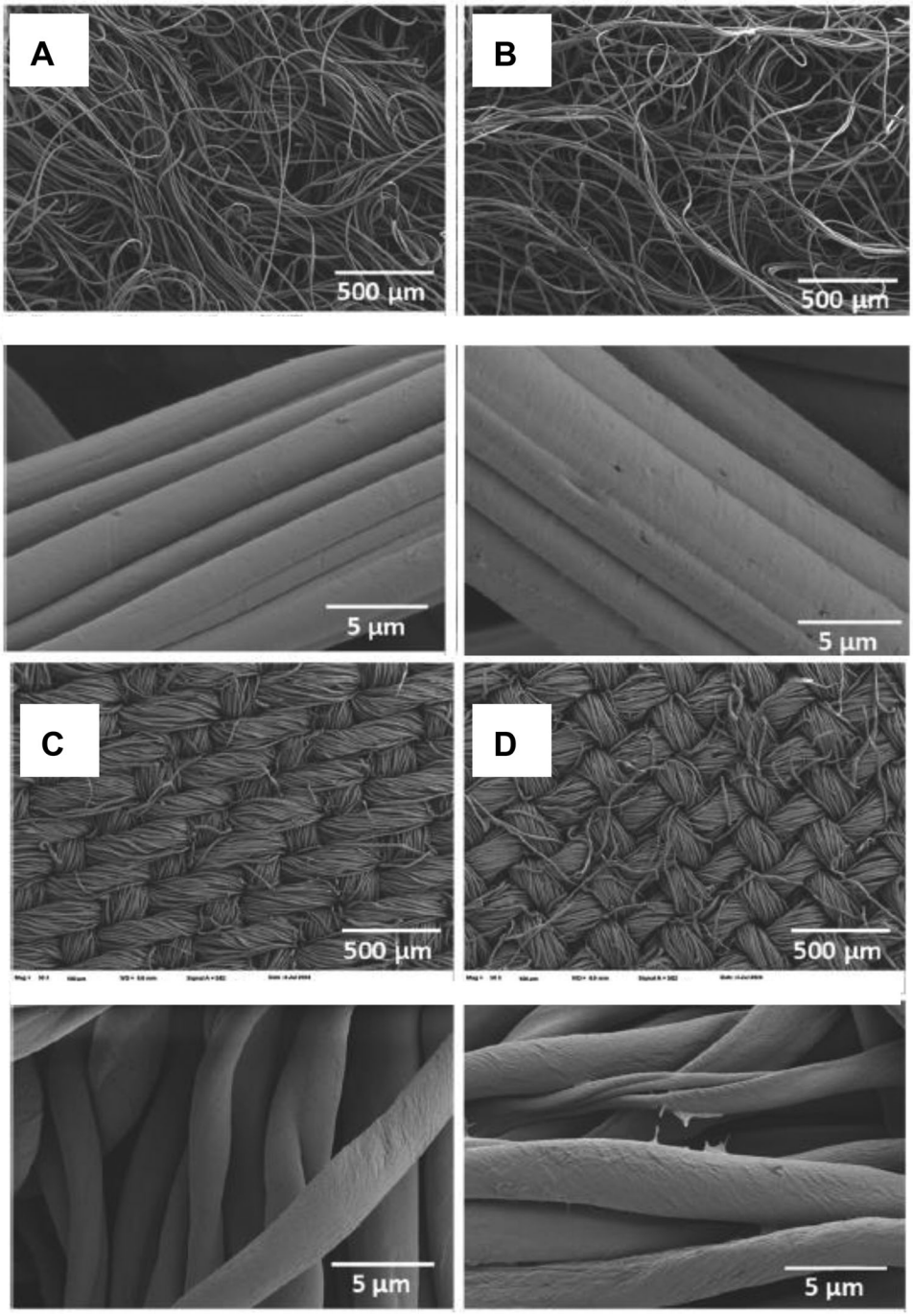

FIGURE 1 | Scanning electron microscopy (SEM), magnification of 50× and 1000× on the surface of (A) viscose reference, (B) chitosan-treated viscose, (C) cotton reference, and (D) GTAC-cationized cotton.

cellulosic materials subjected to modification included commercial woven cotton fabric free of any textile finishing chemicals manufactured by Tan Group Ltd (Turkey) with a mass of ~130 g m^{-2} as well as commercial viscose non-woven free of any textile finishing chemicals manufactured by Suominen Oy (Finland) with a mass of ~50 g m^{-2}. Cationization treatment did not lead to significant changes in morphology on the fibre surfaces (Figure 1). However, viscose non-woven fabric lost its shape and stretched due to the treatments compared to more fixed cotton fabric.

2.1.1 | Covalent Attachment of Glycidyl Trimethyl Ammonium Chloride (GTAC)

Chemical cationization was conducted by immersing the cotton fabric in a reaction mixture of GTAC, isopropanol, water, and NaOH at 60°C for 6 h. After this, the reaction mixture was neutralized with 10% HCl to pH 7–8, followed by extensive washing of the fabric with water and hanging out to dry in a fume hood. Quaternary ammonium groups of GTAC provided the surface a cationic charge independent of pH (see Figure S1).

2.1.2 | Physisorption of Chitosan

Non-woven viscose fabric was immersed into a solution consisting of 5 g L^{-1} chitosan dissolved into acetic acid buffer (0.05 M, pH 5). After 1 h of exposure, the fabric was rinsed with pure buffer solution for 1 h and hung out to dry in a fume hood. Chitosan had a pK_a value of 6.5 induced by primary amine groups, indicating a weak cationic charge at neutral conditions.

2.1.3 | X-Ray Photoelectron Spectroscopy

X-ray photoelectron spectroscopy (XPS) was used to analyze the outermost 10 nm (30–60 atomic layers) of fibre materials. Typically, XPS is used to identify elements and determine relative abundancies of different elemental components in the surface layers of materials. Here, XPS analysis was performed on the viscose reference, chitosan-modified viscose, cotton reference, and GTAC-cationized cotton to evaluate their elemental composition.

Relative proportions of different components of carbon, oxygen, and nitrogen and their binding energies were evaluated to resolve the surface composition of the sample, including the calculation of the extent to which the cationic ammonium or amino groups are present on the surface. Peak fitting of all spectra was done with the CasaXPS software, using peaks of equal full-width-at-half-maximum and standard tabulated chemical shifts for each element (Beamson and Briggs, 1992).

2.2 | Experimental Setup for Capturing *Saprolegnia* Spores

The experiment was performed at the Enonkoski fish farm of Natural Resources Institute Finland (Luke). The experiment took place in two parts: first in spring 2024 and later in fall 2024.

The inlet water was led directly from a nearby Lake Ylä-Enonvesi (area 11.86 km^2, 62°04′00″N, 29°00′00″E) as a flow-through principle 3.5 L s^{-1} (13.6 m^3 h^{-1}; hydraulic retention time: 39 min), without further treatment steps, excluding rough filtration of particles. The inlet water was taken as a mixture from the surface (1 m) and bottom (8 m) layers of the water column. This allows the adjustment of water temperature at the fish farm during warm summer months when high surface water temperatures may occur. In such incidence, the proportion of water from the bottom layer can be increased to maintain suitable water temperature and high enough oxygen content in the water led to the rearing tanks at the fish farm.

The time of the experiment was selected based on the temperature of inlet water, outside temperature, and experience-based knowledge of the most intense time of saprolegniosis incidences in the area. Based on previous years, the time of the most intense saprolegniosis incidences is often at the time of turnover in Lake Ylä-Enonvesi. In 2024, the temperatures remained very low (near 0°C) until the mid of May (Finnish Meteorological Institute, 2024), indicating a late time of year for the spring turnover. Similarly, in fall 2024, the time of experiment was selected based on air temperature and water temperature.

The study followed the protocols approved by the Luke Animal Care Committee, Helsinki, Finland, and EU Directive 2010/63/EU (Council Directive 2010/63/EU) for animal experiments.

2.2.1 | Part I

The experiment conducted for 3 weeks from May 29 to June 11. In total, eight similar flow-through tanks were used for rearing landlocked salmon (*S. salar* m. *sebago*). In each tank, there were 200 individuals of 260 g ± 30 g (biomass of 52 kg $tank^{-1}$, 5.8 kg m^{-3}). The tanks were 12.7 m^2 in size and 8.9 m^3 in volume with the depth of 70 cm.

In total, four types of fabric (1) cotton reference, (2) viscose reference (3) GTAC-modified cotton, and (4) chitosan-modified viscose were studied (4 × 2 tanks), two tanks per type of fabric. The size of a fabric sheet used in the experiments was 29.7 cm × 42 cm, corresponding to a standard A3 sheet size. The fabric was stretched on a wooden frame, two sheets at a time, and placed in the tank water near the source pipe of inlet water due to practical reasons (see Figure S2). The fabric was replaced once a week to avoid excessive saturation and fibre fray.

2.2.2 | Part II

The experiment (part II) was performed in October (from October 14 to 28). The same tanks were used as in part I, but 10 tanks (5 × 2 tanks) were used. In this case, GTAC-modified cotton (one sheet of 29.7 cm × 42 cm), GTAC-modified cotton (two sheets of 29.7 cm × 42 cm), and cotton reference (two sheets of 29.7 cm × 42 cm) were used as described in Section 2.1.1 and replaced once a week. Additionally, formalin (FH 30/4, 30% formaldehyde, Bang & Bonsomer Group Oy, Helsinki, Finland) baths (two times per week, 1.2 L 30% formalin, 1:4000, 0.01%) and reference tanks without treatments were used. For the formalin treatment, water level was decreased from 70 to 30 cm (8.9 m^3→ 3.8 m^3) before the formalin addition. The treatment was continued for 20 min before adding new inlet water and increasing the water level back to 70 cm. During the experiment, there were 60 individuals of 487 g ± 50 g of lake-locked salmon in each tank (biomass 29.2 kg $tank^{-1}$, 3.3 kg m^{-3}).

2.2.3 | Sampling

Samples of water, fabric, and fish were collected once a week before the replacement of the fabric. In total, 500 mL of water was collected in clean high-density polyethene containers and stored at 5°C until delivered for DNA extraction and water quality monitoring. Fish were randomly selected from each tank (fish $n = 1$, tanks $n = 2$) and rapidly euthanized by a sharp blow on the head and stored in an ice bath until delivered for the DNA extraction (described in Section 2.2). Additionally, at the time of

replacement, the used fabric was collected in a zip-lock bag and stored at +5°C until taken for DNA extraction.

2.3 | DNA Extraction

Water, fish, and fabric samples were directly transferred to the laboratory for the DNA extraction in ice bath within 6 h. Water sample processing was started by centrifugation of 100 mL water at 5000 rpm for 30 min. The separated pellet was placed into a 2-mL extraction tube with a 0.8-mL DNA/RNA shield (ZYMO) lysis buffer.

Fish skin samples were scaped with surgery knife and pooled from several places: below the pelvic fin, from the nose, from the side, and from the gills, altogether 0.27–0.46 g of skin and mucus. Fabric samples of 1 cm × 5 cm were cut with scissors and placed in a 50-mL tube filled with phosphate-buffered saline and sonicated for 20 min with an ultrasonic homogenizer (S. Taipale sonicator). The fabric was removed by forceps, and the rest was centrifuged at 2400 *g* for 10 min. The supernatant was removed, and additional centrifugation was done in a 2-mL microcentrifuge tube at 16,000 *g* (relative centrifugal force [RCF]) for 5 min. Finally, 0.8 mL of DNA/RNA shield was added into the tubes.

Then, 0.25 g of UltraClean extraction beads (0.1 mm, Mobio Microbial DNA isolation kit) and 0.5 g Silicon Carbide beads (1.0 mm, BioSpec products #11079110SC) were added to the sample tubes with DNA/RNA shield. The samples were homogenized at 5.5 m s^{-1} (3400 rpm) for 40 s using the Bead Ruptor Elite homogenizer (Omni International) and centrifuged briefly at 10,000 g (RCF) for 4 min. Thereafter, 270 μL of sample was taken into deep well plate for the DNA extraction using the Chemagic Viral DNA kit (Perkin Elmer) for the spring samples (May 29, June 5, June 11) and Chemagic Soil kit for the fall (October 14, 21, 28) samples. The kit was changed due to very low *S. parasitica* genome counts, which led to the need for extra purification of the samples to be able to use bigger amounts of eluate for the PCR reaction. When the new soil kit from Revvity became available during spring 2024, we changed to that, since it had extra purification steps compared to Viral kit, and therefore the kit products were less prone to inhibitory effects in the polymerase chain reaction step. The number of technical replicates was two, used for determining the standard deviations.

For the extraction, 300 μL lysis buffer, 4 μL of polyA RNA, and 10 μL of proteinase K, all provided by the kit, were added, and the purification and elution of the DNA were done using the chemagic 360 automate (Perkin Elmer). After the extraction, the sample was dissolved in 80 μL of elution buffer. Part of the sample was re-purified using SparQ-magnetic beads (Quantabio); 60 μL of DNA and 60 μL of magnetic bead solution were used for the purification. The final elution was performed with 25 μL of deionized water.

2.4 | Quantitative Polymerase Chain Reaction

The quantification of *S. parasitica* DNA was performed with a quantitative polymerase chain reaction (qPCR) assay following the protocol of Korkea-aho et al. (2022). The qPCR reactions were run with the BIORAD CFX Real-Time PCR System (Bio-Rad) with primers and probe amplifying the *S. parasitica* ITS2—28S region with primer-F (5′-AGAGCAAATCGCGGTAGTTT-3′), primer-R (5′-AGAAATGCACCAGCATACCA-3′), and probe-R (5′-FAM-TGCCTTGTACTTTGACAA CAGACTCGC-BHQ2-3’) (Metabion). For pure culture isolates, standard genomic DNA of *S. parasitica* pure culture was obtained from the laboratory of Dr. Tiina Korkea-aho (Finnish Food Authority, Finland). The final volume of the PCR reaction was 22 μL, with 11 μL of Bio-Rad iTaq Universal Probes Supermix (2×) (BioRad), 5 μL of DNA template, 400 nM of primers, 200 nM of probes, and PCR-grade water. The PCR protocol was 5 min at 95°C followed by 45 cycles: 10 s at 95°C and 30 s at 60°C.

A standard curve was made from DNA of *S. parasitica* pure culture, which has a genome size of 63 Mb, accounting for 15.5 genomes per picogram DNA. Technical limit of detection (LOD) in the PCR-reaction was 250 fg μL^{-1} for *Saprolegnia* DNA template (3.6 genomes μL^{-1} template, 5 μL of extract was used). Theoretical LOD of the process was 36 *S. parasitica* genomes per 100 mL sample, when back-counted by known volumetric losses in the process (genomes per μL DNA extract)*800/270*80/60*25/50*25/5 (Figures S3–S4).

2.5 | Water Quality

The total nitrogen (0–0.8 mg L^{-1}, Procedure 8038 Nessler), nitrite (0.015–0.6 mg L^{-1}, LCK341), nitrate (5–35 mg L^{-1}, LCK340), phosphate (0.05–1.5 mg L^{-1}, LCK349), sulphate (40–150 mg L^{-1}, TNT864), and chemical oxygen demand (COD; 5–60 mg L^{-1}, LCK 1414) were analyzed using quick spectrophotometric tests for DS 3900 (Hach, Loveland, USA). The water pH and alkalinity (88.3–113.1 mg L^{-1}) were measured by a standard titration method ISO 9963-1:1994 (TitraLab AT1000; Hach) and turbidity (5.5–6.6 NTU, Nephelometric Turbidity Units) with Hach 2100q Turbidimeter (Hach).

2.6 | Statistical Analysis

Statistical analyses were performed with IBM SPSS Statistics for Windows (released 2020; version 27.0.1.0; IBM Corporation, Armonk, NY, USA). A linear mixed model analysis was performed to study the statistical difference in water quality parameters and the *S. parasitica* spores. The confidence interval was set at 95%.

3 | Results

3.1 | Elemental Composition of Capturing Materials

In the XPS analysis, the results of the fitting of nitrogen components to the N 1s spectra can be found in the first two columns of Table 1, indicating that the samples likely contained nitrogen in the form of both amines and protonated amines. The third column shows the total amount of nitrogen compared to all of the elements in the sample, while the fourth column gives the

TABLE 1 | Relative amounts of the different components of nitrogen, as compared to the total amount of nitrogen in the samples (R-NH_2, %; NR_4^+, %), total nitrogen concentration (N, %), and ratios between nitrogen and carbon amount (N/C, %), and nitrogen and unit cell. unit (N/cell).

Sample	N (R-NH_2) (%)	N (NR_4^+) (%)	N (%)	N/C (%)	N/cell. unit
A	100.00	0.00	0.09	0.13	0.01
B	96.51	3.49	0.15	1.88	0.13
C	98.41	1.59	0.07	0.82	0.05
D	36.78	63.22	0.42	6.41	0.62

Note: A: viscose reference, B: chitosan-modified viscose, C: cotton reference, and D: GTAC-cationized cotton.

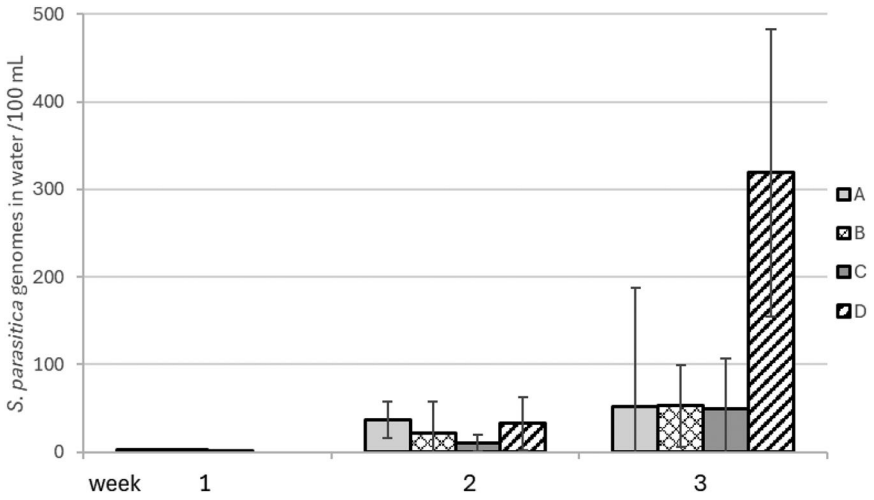

FIGURE 2 | Genomes of *S. parasitica* in water (per 100 mL) after 1, 2, and 3 weeks of the experiment in May–June 2024, tank replicates $n = 2$. (A) cotton reference, (B) viscose reference, (C) GTAC-modified cotton, and (D) chitosan-modified viscose. No statistically significant differences in *S. parasitica* genomes were found between different types of fabric ($p > 0.05$; Supplementary Table S5).

percentage of nitrogen compared to the amount of carbon in the sample. The last column gives an estimate of the amounts of nitrogen-containing groups or sub-units per unit cell of cellulose in the sample.

For GTAC-modified cotton, the elemental ratio of nitrogen to cellulosic unit (corresponding to the degree of substitution in the surface of the material) was 0.62 as detected with the XPS, indicating relatively high cationic charge with six ammonium groups per 10 glucose units. For chitosan-modified viscose, the elemental ratio of nitrogen to cellulosic unit was 0.13 as detected with the XPS, indicating moderate cationic charge with about 1 amine group per 10 glucose units.

Relative amounts of carbon and oxygen are presented in the Supporting Information (Tables S1 and S2), C 1s and N 1s spectra in Figure S5, while survey spectra are shown in Figure S6.

3.2 | Part I

Water quality parameters were monitored during the experiment (Table S3), but no statistically significant differences ($p > 0.05$) were observed between the treatments (Table S4).

In water, the number of *S. parasitica* genomes increased during the 3 weeks of the experiment (Figure 2). After week 1, genomes were only scarcely observed in all systems. After week 2, genomes were more readily present in water, showing minor differences between the systems. Finally, after 3 weeks, the highest number of genomes was observed in the systems with chitosan-modified viscose fabric.

During the first 2 weeks of the experiment, the highest number of *S. parasitica* genomes (per gram of fabric) was detected in GTAC-modified cotton (Figure 3). However, after week 3, the highest number of genomes was detected in chitosan-modified viscose. At week 3, both modified fabrics (C and D) showed a higher number of genomes with significant differences compared to the reference fabrics (A: cotton reference, B: viscose reference). Furthermore, viscose non-woven reference seemed slightly more sensitive to the presence of *S. parasitica*, showing higher captured amount already at week 2 compared to the woven cotton reference.

In the case of fish, *S. parasitica* genomes were found only after 3 weeks of the experiment (Figure 4). The highest number of genomes was found in systems with GTAC-modified cotton. Significantly lower numbers were found in systems with cotton and viscose references. Especially in systems with GTAC-modified

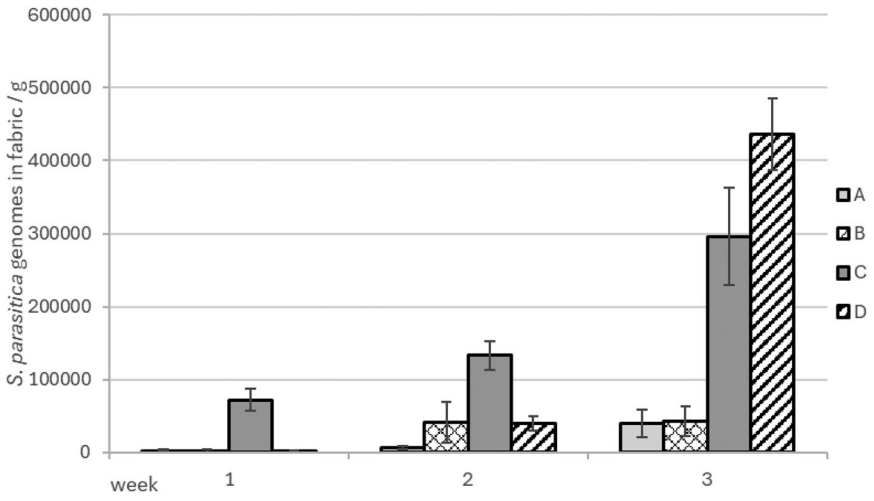

FIGURE 3 | Genomes of *S. parasitica* in fabric (per gram) after 1, 2, and 3 weeks of the experiment in May–June 2024, tank replicates $n = 2$. (A) cotton reference, (B) viscose reference, (C) GTAC-modified cotton, and (D) chitosan-modified viscose. No statistically significant differences in *S. parasitica* genomes were found between different types of fabric ($p > 0.05$; Supplementary Table S5).

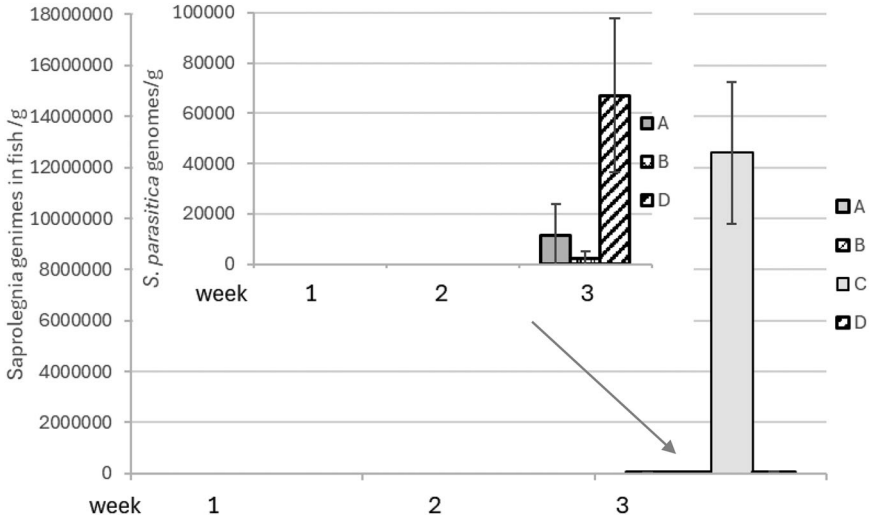

FIGURE 4 | Genomes of *S. parasitica* in fish (per gram) after 1, 2, and 3 weeks of the experiment in May–June 2024, fish $n = 1$, tank replicates $n = 2$. (A) cotton reference, (B) viscose reference, (C) GTAC-modified cotton, and (D) chitosan-modified viscose. No statistically significant differences in *S. parasitica* genomes were found between different types of fabric ($p > 0.05$; Supplementary Table S5).

cotton and chitosan-modified viscose, water mould occurrences in fish (grey hyphae on skin of fish) were observed also via visual inspection.

3.3 | Part II

Water quality parameters were monitored during the experiment (listed in Table S6). No statistically significant differences ($p > 0.05$) were observed between the treatments (Table S7).

In part II, *S. parasitica* genomes were not detected either in water or in fish samples during the experiment, leaving undetected any differences between formalin treatments, systems with reference or modified material, and the controls. However, *S. parasitica* genomes were detected in the studied materials GTAC x1, GTAC x2, and in the unmodified cotton reference (Figure 5) but without statistically significant differences between the materials ($p > 0.05$).

4 | Discussion

4.1 | Material Properties and Binding

Cationic modification increased the capturing ability of cellulosic fabrics towards *S. parasitica* spores, indicating electrostatic attraction between the cationic fabric and the anionic spores to play a critical role in the capturing process. Of the two routes for the preparation of cationic cellulosic fabric in part I of the study, woven cotton chemical modified with GTAC proved somewhat more effective than non-woven viscose modified via

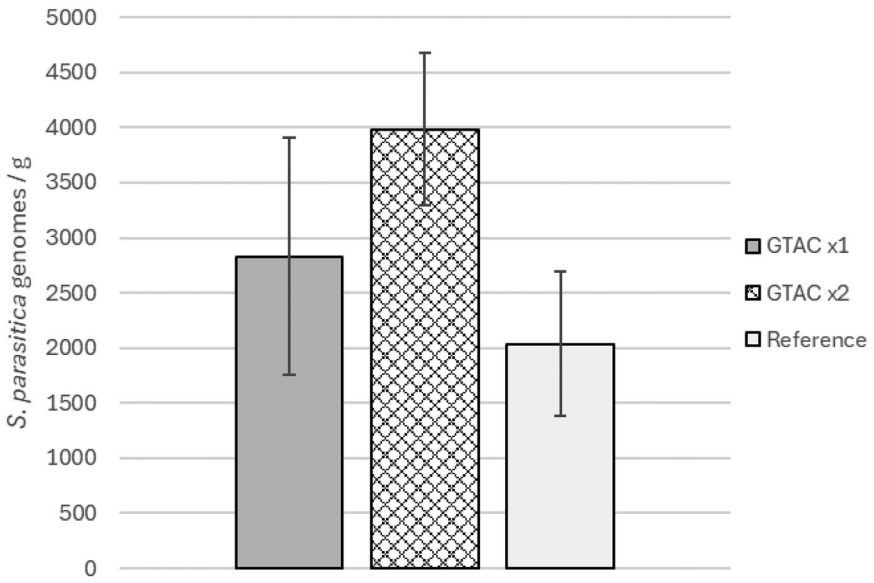

FIGURE 5 | Genomes of *Saprolegnia parasitica* in fabric (per gram) on average per week after 3 weeks of the experiment in October 2024 (tank replicates $n = 2$). GTAC ×1: GTAC-modified cotton (area × 1), GTAC ×2: GTAC-modified cotton (area × 2), and Reference: unmodified cotton (area × 2). No significant differences were found between different types of fabric ($p > 0.05$).

the physisorption of chitosan. The efficiency of the cationization method for the two different capturing materials was likely to affect the capturing efficiency. The GTAC-modified material is likely to induce stronger electrostatic attraction by the significantly, around an order of magnitude, higher charge density of the material (0.62 cationic quaternary ammonium groups per glucose ring) compared to that reached via the chitosan-modified material (0.12 primary amine groups per glucose ring, of which only a faction are protonated at the pH condition of the study), as indicated by the XPS analysis. The significance of the degree of cationization is further supported by the fact that the increased amount of cationized fabric in the system, in part II of the study, resulted in an increased number of captured spores.

The utilization of the electrostatic attraction for enrichment of material within biological systems is not unprecedented. Cameron and Carlile (1977) has already suggested a mechanism of cation interaction with negatively charged oomycete membrane. The so-called positive chemotaxis leads to the attraction of zoospores to various organic compounds, which increases the chances of finding a suitable host (Duan et al. 2018). These results showed the effects of zoospores' ability to seek towards a suitable host, for example, *S. parasitica* was attracted to fish tissue (El-Feki et al. 2003).

Electrostatic effect reaches only some tens of nanometres distance from the surface at maximum, with the distance decreasing with decreasing water purity (Israelachvili 2011). However, the functionality of relatively short distance has a far-reaching effect over time, as the water flows in a fish tank, enabling enrichment of spores at the fabric surface.

Followed by their enrichment near the surface, the actual attachment mechanism of the spores onto the capturing material is likely to involve other components than electrostatic attraction. Zoospores of *S. parasitica* are known to include bundles of hairs, composed of glucose and mannose (Burr and Beakes 1994; Rezinciuc et al. 2018), with hooks at their tips as presented in Rezinciuc et al. (2018). The bundles of *S. parasitica* were on average 10 μm in length with the hooks of 0.7 μm (Rezinciuc et al. 2018). Hairs and bundles are not passive as proposed by Wood et al. (1988) but play a role in attachment and increase the attachment efficiency of *S. parasitica* spores to a potential host (Beakes et al. 1994; Rezinciuc et al. 2018).

Moreover, the study of Rezinciuc et al. (2018) showed that the strength of cysts attachment on *Saprolegnia* spp. correlated with the length of bundles. Threefold differences were observed in attachment strength between long-haired and hairless cysts. However, Burr and Beakes (1994) and later Rezinciuc et al. (2018) suggested that stickiness properties may be involved in the attachment of *S. parasitica* cysts. This may also play a role in the spores´ attachment onto the surface of the capturing material.

In this study, some general assessment on the role of mechanical binding could also be made as it appears to play a role besides the electrostatic effect. Regarding the unmodified reference fabrics, the slightly higher sensitivity of the non-woven viscose for the capture of spores compared to that of the tightly bound woven cotton may indicate that the looser structured fabric offers morphologically better accessibility and more sites for mechanical attachment of spores via hooked hairs. Although the significance of this effect for the overall spore-capturing mechanism seems secondary compared to that of the electrostatic effect, it is likely that the mechanical accessibility of the capturing material affects the mechanical attachment and thus plays a role in the capturing process. The contribution of the morphology of the capturing material on the attachment of spores should be addressed in more detail.

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compared to that of the tightly bound cotton woven might indicate that the looser structured fabric offers morphologically better accessibility and more sites for mechanical attachment of spores. The significance of this effect for the overall spore capturing mechanism in this study, however, seems secondary compared to that of the electrostatic effect. Later on, the binding mechanisms need to be studied in more detail if the binding occurs only due to electrostatic forces between cationic and anionic surfaces or if the attachment ability of hooked hairs plays a role.

In this study, the highest numbers of *S. parasitica* genomes were detected in systems with chitosan-modified viscose. This is in accordance with the spore numbers in water; the highest numbers were in systems with chitosan-modified material. As far as we are aware, chitosan does not induce growth of *S. parasitica* spores. On the contrary, studies (e.g., Muzzarelli et al. 2001; Silva-Castro et al. 2018) have shown that chitosan and its derivatives can act as oomycete inhibitors. Here, the number of fish taken for analysis was low, suggesting that sampling may not have been representative. The sampled fish in systems with chitosan-modified material clearly suffered from saprolegniosis, likely caused by the high number of genomes detected by the qPCR. Therefore, we cannot conclude that chitosan was the cause of high number of *S. parasitica* genomes. Instead, chitosan-modified material was not fully able to prevent saprolegniosis occurrences.

In part II, a higher number of *S. parasitica* genomes were observed in the GTAC-modified material compared to the unmodified cotton (reference). Additionally, the doubled surface area (A3-sized sheets) collected more *S. parasitica* genomes than one sheet of GTAC-modified cotton, although without statistical significance. This suggests the importance of finding an optimum amount of material per genome count and inlet water volume. This way, we could offer enough active surface for capturing enough *S. parasitica* genomes and reducing the pressure for saprolegniosis occurrences.

4.2 | Sampling and Detection of *S. parasitica* Genomes

One of the obstacles in culture-based detection methods is the limited sample size, while in sampling for qPCR, several litres of sample can be filtered, which makes the detection of the pathogen in environmental samples more plausible (Kokea-aho et al. 2022; Härkönen et al. 2024). However, when using large amounts of water, the accumulation of inhibitors in the sample is also more likely. In our study, only 500 mL of water was collected, and even less was used for the qPCR, decreasing the likelihood for the detection of *S. parasitica* genomes.

For this study, a preliminary study was conducted regarding the filtration. Unfortunately, a large amount of aqueous sample and a large filter were needed to detect the rarely occurring *S. parasitica* genomes. To avoid the observed difficulties and sources for inaccuracy with multiple filtration steps in the method, centrifugation was chosen to process the samples. Indeed, it showed good results and was less prone to contamination.

In previous studies, fish tissue samples have been collected for the extraction with Qiagen DNeasy Blood & Tissue kit (Kokea-aho et al. 2022; Härkönen et al. 2024). In this study, tissue samples were scraped and sonicated with an ultrasonic homogenizer. Additionally, extraction from the fabric samples was modified for the purpose of this study. A negative control was used in both studies. Here, the sampling of fish was successful in part I, but it may have suffered from inhibition in part II. However, the inhibition was not possible to test or confirm during the study period.

4.3 | Conditions and Water Quality

The skin of fish plays a crucial role against oomycete infection, as it is the first point of contact (Bruno and Wood 1999; Duan et al. 2018). Physically injured, stressed or infected fish are more susceptible to oomycetes infection (Pickering and Willoughby 1982; Lindholm-Lehto and Pylkkö 2024). For farmed fish, physical injuries from mechanical damage can occur due to high stocking densities or injuries from farming equipment. In our study, the stocking densities were kept low (biomass ranged from 3.3 to 5.8 kg m^{-3}), and equipment that might harm the fish were not used in the rearing tanks to prevent any mechanical injuries on fish skin.

The organic material including humic acids may be transported from the soil and from the water body, increasing the content of humic substances in the inlet water of Enonkoski fish farm. In the report by Hentinen and Pursiainen (2004), there are peat lands, which may act as a source of humic matter drainage. Additionally, the forests have largely been drained, which leads to emissions of particulates and nutrients into the water bodies, including Ylä-Enonvesi (Hentinen and Pursiainen 2004).

In lake Ylä-Enonvesi, the runoff peaks in the spring when the snow melts (April–May 2024, 7 mm day^{-1}; 0–1 mm day^{-1} other months in 2024; Vesistöennusteet 2025) up to sevenfold volumes. The volumes may change between years, for example, based on snow volumes in the area. In our study, the experimental part I occurred in May–June 2024 after the highest runoff. However, we observed mostly low turbidity (0.7–2.8 NTU, part I and 0.5–2.4 NTU part II), and only once a peak of 4.05 NTU. Based on Pavić, Grbin, Gregov, et al. (2022), increased humic acids in water resulted in increased sporulation of *S. parasitica*. While the relationship between saprolegniosis occurrences and high organic and humic matter has been suggested (Pavic, Grbin, Gregov, et al. 2022), other studies have suggested the inhibition of saprolegniosis due to humic material with high aromaticity and high C:H and C:CH_2 ratios (Meinelt et al. 2007). In this study, saprolegniosis was observed at the fish farm despite of low turbidity, suggesting low organic matter content.

Furthermore, it has been suggested (Chiasson et al. 2023) that the presence of humic substances (humic acid, fulvic acid, humins) might reduce saprolegniosis occurrences and increase the defence mechanism against *Saprolegnia* in rainbow trout eggs and larvae (Schreckenbach et al. 1994; Meinelt et al. 2008; Lieke et al. 2020). Although Chiasson et al. (2023) reported promising results in preventing saprolegniosis, the disease was observed at the fish farm conditions of our study when humic material was

readily present. However, adult fish were studied in our study, not eggs.

Recently, Pavić, Grbin, Gregov, et al. (2022) reported that dissolved organic carbon (DOC) and humic acids positively correlate with the sporulation intensity of *S. parasitica*. Humic acids are widespread substances, which contribute to the DOC concentration and are common in the aqueous environment in the Nordic countries and in Finland (Lindroos et al. 2020). In our study, DOC was not measured, but COD and turbidity were used to monitor changes in organic matter content. No significant differences were not found in turbidity or in COD between systems in part I or part II. Saprolegniosis was observed during the experiment, although the organic matter contents, including humic material, was low. This suggests that other factors contribute to saprolegniosis occurrences.

Water temperature may not only affect host immunity but contribute to the abundance of *S. parasitica* and its mycelial growth and spore production (Matthews 2019; Pavić et al. 2021). This may partly explain the differences in detected *S. parasitica* spores between part I (20°C) and part II (10°C). For example, the highest mycelial growth of *S. parasitica* on fish skin has been observed at temperatures between 15–25°C (Matthews 2019). Rapid changes in temperature may lead to stress reaction and immunosuppression fish, more likely leading to saprolegniosis occurrences (Duan et al. 2018; Härkönen et al. 2024). Härkönen et al. (2024) suggested that potential environmental sources for variation in *S. parasitica* load, such as water quality (including nutrients and minerals), organic load (humus), and pH (Pavić, Grbin, Hudina, et al. 2022), should be considered.

Our results showed low or moderate organic contents, neutral or mildly acidic pH, and low concentrations of nitrogen compounds, but still saprolegniosis occurrences were observed. Typically, mycelial growth and zoospore production are favoured by acidic conditions (Kitancharoen et al. 1996; Tedesco et al. 2022). Additionally, high contents of organic matter and nitrogen compounds have been associated with saprolegniosis occurrences (Pavić, Grbin, Hudina, et al. 2022; Tedesco et al. 2022), but neither seemed to explain the saprolegniosis occurrences or showed significant differences between the systems.

5 | Conclusions

It seems reasonable to assume that electrostatic attraction between surface-modified cellulose material with cationic groups and the *S. parasitica* spores enhanced the enrichment of spores at the cellulosic surface. In the actual binding process, mechanical attachment by the spores' hooked hairs is also likely to play a role. Further studies are required to distinguish between the contribution of these two different factors to the binding mechanism.

The results of the study showed significantly better attachment of *S. parasitica* spores on the modified cellulose materials compared to the unmodified cellulose. Furthermore, *S. parasitica* was observed also in water and on fish skin. Overall, GTAC-modified cellulose showed the best results with the highest number of spores attached to the material.

Increased content of humic compounds was observed in water and also in the inlet water of Enonkoski fish farm, especially in fall (experimental part II). This may have inhibited the qPCR analysis because *S. parasitica* genomes were not detected in water or in fish by qPCR analysis, although visual observations supported the occurrence of saprolegniosis at the fish farm. Unfortunately, this did not allow us to detect differences between formalin-treated, GTAC-modified, reference, and untreated systems. Even in this case, the highest number of genomes was detected in the GTAC-modified cotton.

To conclude, the presented experiments showed us that *S. parasitica* spores can be captured by modified cellulose-based material and the concept of capturing *S. parasitica* spores works. Further studies are required to optimize the content of the material, process solutions how to most efficiently introduce the material to a flow-through fish farm, and how often material needs to be changed among many other parameters.

Author Contributions

The study was designed and implemented by Petra Camilla Lindholm-Lehto. Katri S. Kontturi and Hannes Orelma designed and provided the tested materials. Kristoffer Meinander performed the XPS analyses. The manuscript was drafted by Petra Camilla Lindholm-Lehto and Kristoffer Meinander. Hannes Orelma carefully examined and reviewed the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.

Supplementary Material: aff270172-sup-0001-SuppMat.docx