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What's new in the hatchery? Humic acid as a microbial control agent

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Cultured fish species are increasingly exposed to fungi and bacteria in the rearing environment which often causes disease and mortality. Aquaculture producers rely on the use of chemical therapeutics to combat the effects of fungi and bacteria which may have negative consequences for fish, human and environmental health. In the hatchery environment, managers seek solutions which are both cost-effective and sustainable.

Egg treatment in hatcheries

Several antifungal agents are commonly used during the incubation of salmonid eggs. These agents may vary depending on preferences, regional regulations, and specific hatchery practices. Antifungal treatments play a significant role in promoting sustainable hatchery production in several ways. Primarily, fungal infections can pose a significant threat to fish eggs and larvae in hatcheries and may contribute to disease. By using antifungal treatments, the risk of fungal infestations can be reduced. This prevention helps maintain healthier fish populations and minimizes the need for disease treatment, which can be costly and time-consuming.

The use of antifungal treatments can also increase hatch rates. Fungal infections can negatively impact egg viability and hatch rates. Treating the eggs with antifungal substances helps protect them from fungal pathogens, ensuring a higher percentage of eggs successfully hatch. Improved hatch rates contribute to higher production yields and more efficient use of resources in the hatchery.

Fungal infections may also weaken fish embryos and fry, making them more susceptible to other diseases and stressors. By implementing antifungal treatments, the overall health of the fish is improved. Healthy fish have a better chance of surviving, growing, and reaching market size, which contributes to sustainable aquaculture production.

In Ontario, Canada, the most common treatment during the incubation of rainbow trout eggs is formalin (formaldehyde). Formalin is a widely used antifungal agent in aquaculture. It is effective against various fungi and has been used for many years in egg disinfection. Formalin treatments are typically performed as short-term baths or continuous low-level exposure to control fungal infections on eggs. It is important to handle formalin with care as it is a hazardous substance and requires appropriate safety measures.

Historically, malachite green, a synthetic dye with antifungal properties, has also been used for the treatment of fungal infections in fish eggs. Malachite green can be applied as a bath or used in egg suspension during incubation. However, its use is regulated or banned in some countries due to potential environmental and health concerns. Hydrogen peroxide is a mild antifungal and disinfecting agent which may also be used to control fungal growth on rainbow trout eggs. Hydrogen peroxide is typically applied as a bath or in low concentrations as an egg disinfectant. This product must be used with care because excessive concentrations will harm the developing embryos. Finally, copper sulfate is also an effective antifungal and bactericidal agent used in aquaculture and may be used as a treatment for fungal infections on trout eggs. However, its use requires caution as excessive copper concentrations can be toxic to fish and other aquatic organisms. All these products have varying consequences for fish, human and environmental health.

Solutions from nature

The balancing act of managing the health of incubating eggs while also limiting the exposure of fish, humans, and the environment to potential chemical irritants, has many hatchery managers seeking solutions from natural sources. The development of nontoxic and environmentally friendly products to safely disinfect salmonid eggs during incubation would reduce risks to human and environmental health.

One potential solution is humic acid, a natural organic compound that is derived from the decomposition of plant and animal matter over a long period of time. Humic acid is commonly found in soils, peatlands, and freshwater environments. Humic acid forms because of the breakdown and transformation of organic materials, such as leaves, wood, roots, and other organic debris, by microorganisms and geological processes.

Humic acid is primarily known for its applications in agriculture and soil management due to its ability to improve soil fertility, nutrient uptake, and water retention. However, it is also used in several other industries for disinfection purposes and other applications. Humic acid can be used in water treatment processes, particularly in the removal of heavy metals and other contaminants. Its chelating properties enable it to bind with metal ions and facilitate their removal from water, helping to purify and detoxify water sources. Humic acid has been employed in the remediation of contaminated soils and water bodies because it can aid in the removal or immobilization of various pollutants, including heavy metals, pesticides, and organic compounds, thereby assisting in the cleanup and restoration of polluted environments. Humic acid may also be added to livestock and poultry feed as a supplement. It has been associated with improved nutrient absorption, gut health, and immune function in animals. Humic acid is generally considered safe when used as directed and in appropriate concentrations.

In the context of aquaculture, humic acid has been utilized for various purposes, including water quality management, disease prevention, and stress reduction in fish. It can help maintain favorable water conditions, promote the growth of beneficial microorganisms, and enhance the immune response of fish, contributing to healthier and more resilient aquaculture systems.

Humic acid in practice

We aimed to test the effect of humic acid as a treatment to reduce fungal and bacterial infections to increase survival during the incubation of rainbow trout (*Oncorhynchus mykiss*) eggs. To do this, we used a peristaltic pump to continuously expose rainbow trout eggs in stacked incubating trays to a low-level (5 mg/L) of humic acid (trade name: AC Aqua, MTS Environmental Inc., Exeter, ON, Canada) from fertilization until hatch. To assess the impact of humic acid, we counted the number of eggs going into the incubator and counted them again at the eyed stage and at hatching. Additionally, we collected water samples from the incubators and used 16S ribosomal RNA (rRNA) sequencing to identify and compare bacterial diversity between the eggs treated with humic acid and the eggs incubated in regular groundwater (control).

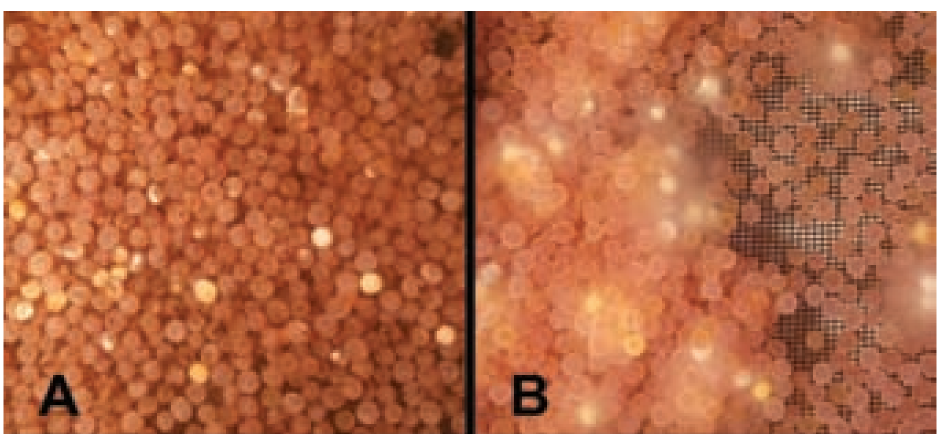

Figure 1. Incubating rainbow trout eggs at 35 days post fertilization incubated at 9°C in stacked incubator trays exposed to groundwater (control) and humic acid at 5 mg/L (treatment). Image A (left) has no visible evidence of fungus whereas image B (right) shows clusters of white fungal mycelium.

In the absence of chemical or manual manipulation to reduce fungus growth, we expected the egg mortality in the control groups would be high. We predicted that exposure to humic acid in the water would reduce the growth of the fungal mycelium. The results were better than we expected, exposure to humic acid in the water eliminated

Table 1. Percent survival (± standard deviation) of rainbow trout eggs collected weekly for three weeks at the eyed stage (24 days post fertilization) and hatch (42 days post fertilization) incubated at 9°C in stacked incubator trays exposed to groundwater (control) and humic acid at 5 mg/L (treatment). Different letters in the same column indicate significant differences within groups $p \leq 0.05$.

Egg collection	Group	Mean survival to eyed stage (%)	Mean survival to hatch (%)
Week 1	Control	84.0 ± 5.3	68.8 ± 11.8
	Treatment	90.7 ± 1.1	80.9 ± 1.7
Week 2	Control	82.8 ± 2.5	59.4 ± 5.6
	Treatment	97.7 ± 0.5	74.7 ± 16.5
Week 3	Control	78.5 ± 4.6	38.5 ± 11.8
	Treatment	98.0 ± 0.5	75.9 ± 9.7
Week 4	Control	88.9 ± 4.6[a]	55.5 ± 16.1[a]
	Treatment	95.5 ± 3.6[b]	77.2 ± 10.4[b]

observable fungus and resulted in significantly improved survival compared to the control groups in the incubator trays (Fig. 1).

Specifically, the survival in the eggs treated with humic acid (mean 95.5 ± 3.6 %) was significantly greater than the controls (mean 88.9 ± 4.6 %) at eye-up which occurred at 24 days post fertilization (dpf). We saw an even more pronounced effect at hatch (42 dpf), the survival in the eggs treated with humic acid (mean 77.2 ± 10.4 %) was significantly greater than the control group (mean 55.5 ± 16.1 %; Table 1).

Additionally, the humic acid treatment was found to reduce the bacterial diversity compared to eggs incubated in groundwater and altered the bacterial composition after 20 days of continuous exposure. Finally, the humic acid treatment increased the abundance of bacteria associated with healthy fish eggs and decreased the abundance of known bacterial pathogens, such as *Flavobacterium* and *Aeromonas*.

A sustainable future

As water temperatures continue to rise, hatchery producers are likely to experience more pressure due to fungal and bacterial pathogens in their culture facilities. Overall, antifungal treatments contribute to sustainable hatchery production by improving fish health, increasing hatch rates, reducing disease risks, and promoting responsible resource management. By implementing these treatments, hatcheries can operate more efficiently, minimize environmental impacts, and support long-term viability in the aquaculture industry. We found that humic acid is a viable alternative for hatchery producers to use as a water treatment to reduce bacterial and fungal pathogens to improve the survival of rainbow trout eggs in incubator trays. Not only was this product effective in eliminating fungal growth, but additional benefits were also observed in the microbial community in the incubator trays. By moving away from chemical products, such as formalin, and implementing natural antifungal substances like humic acid, hatcheries can minimize their chemical footprint and adopt more sustainable practices.

Reference:

Chiasson M, Kirk M and Huyben D (2023). Microbial control during the incubation of rainbow trout (*Oncorhynchus mykiss*) eggs exposed to humic acid. Front. Aquac. 2:1088072. doi: 10.3389/faquc.2023.1088072

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