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Post malachite green: Alternative strategies for fungal infections and white spot disease

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Background

Malachite green oxalate has been used for decades in fish farming to treat fish and eggs successfully against *Saprolegnia* infections and fish juveniles against protozoan *Ichthyophthirius multifiliis*. According to the Act of European Council (2377/90) those drugs used in animal production which don't have the MRL-value (maximum residue limit) are not allowed to use since 1.1.2000. Malachite green is one of those drugs without the MRL-value and due to its carcinogenic indications it will not receive this value. Alternative chemicals have been tested and used in many countries with varying success, and problems as well as losses of fish and eggs has been reported. Practical guidelines for aquaculture are still lacking. The purpose of this round table discussion was to round up experts and expertise in this field in order to share experiences and research results.

Results of the ICH questionnaire

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The organisers of the workshop had performed a questionnaire, which had been sent to eleven branch officials of the EAFP. The branch officials had sent it further to fish disease professionals, who had practical experience of treating white spot disease (ICH) during the latest years. The questions were designed to give information especially on the use of alternatives for Leteux-Meyer mixture (= malachite-formalin). The results were based on the practical experience of ICH treatment in total at least ca. 30 fish farms.

The experience came mainly from treatment of salmonids (mentioned in seven answers), but common carp, Japanese flounder, roach, perch and tench were also mentioned in one answer each. The respondents regarded the density of fish being the most important predisposing factor for white spot disease, water temperature and slow flow of water being the next important ones. The most widely used alternative for Leteux-Meyer had been recurrent short baths with formalin (concentrations 60-250 ppm, treatment for 60-20 minutes at each time). This had been applied for salmonids and was regarded as good as Leteux-Meyer mixture in one answer and worse in six. Two respondents claimed worse results than with Leteux-Meyer mixture with two other short baths using either sodiumcarbonateperoxyhydrate (60-90 ppm, 30-60 minutes, daily for 4-6 days) or a mixture of acetic acid, peracetic acid and hydrogen peroxide (Detarox, 10 ppm, 25-40 min-

Compound	Treatment (ppm)	Regime	% trophont reduction
Amprolium hydrochloride	104	10 days prior to infection	c. 63%
	75		c. 62%
	63	10 days once infected	c. 75%
Clopidol	65	10 days prior to infection	c. 35%
	92		c. 23.2%
Salinomycin sodium	47	10 days once infected	c. 94%
Bronopol (Pyceze)	100 (30 mins)	10 times over 10 days	significant effect in 50% of tanks
	100 (30 mins)	10 times over 10 days as trophonts exit	c. 17%
Chloramine T	100 (30 mins)	4 times over 10 days	significant effect in 50% of tanks
	100 (30 mins)	10 times over 10 days as trophonts exit	97.3% 90.5%

Table 1. Shinn et al., control of I. multifilis

utes, 2nd treatment after 4-7 days). These had been used for salmonids as well as copper sulphate dips (250-500 ppm, 30 seconds) and metronidazole in feed (7.5 mg/kg fish/day for 7 days). The last two had given as good or better result as Leteux-Meyer mixture. Copper sulphate is, however, relatively toxic for fish and dips labour intensive and time consuming to use. Metronidazole is not allowed to be used for production animals e.g. in the EU any more.

For other species than salmonids continuous sodium chloride baths (0.3-1.0 % for 3-11 days) was mentioned to be better than Leteux-Meyer mixture for common carp as well as 3 days-continuous "copper ionic treatment" for Japanese flounder. The need for medical treatment had been reduced by measures opposite to the predisposing factors. One respondent claimed to have done this also by regular vitamin C in feed (for one week/month, 1-3g/kg feed) and another with immunostimulants.

Experiments in Scotland: The assessment of novel chemotherapeutic compounds for the control of Ichthyophthirius multifiliis (Fouquet, 1876) (Ciliophora)

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Ichthyophthirius multifiliis infection is an increasing problem in freshwater culture and its control is notoriously difficult. The prohibition of the use of malachite green as a treatment for food fish and the low efficacy of current permissible alternatives make the search for alternative compounds a matter of urgency. The aim of the project was therefore to assess the efficacy of a range of bath and in-feed chemotherapeutants *in vivo* against the ciliate, *I. multifiliis,* using laboratory and field trials.

A series of replicate, 10 litre flow-through tanks each containing 10-15, *O. mykiss* (5g) were conditioned for 2 weeks prior to the start of each treatment trial. On infection day, fish were exposed to a pre-determined number of theronts (c. 2000 theronts per fish) for c. 2 hours and later randomly allocated equally between the test and control tanks. The chemotherapeutic efficacy of 6 in-feed, 3 bath and 5 feed additive compounds was assessed from trophont numbers which were then subjected to statistical analysis.

A total of 175 *in vivo* tests were conducted and a summary of the most efficacious doses and methods of compound administration are given in Table 1.

Conclusions
The two bath treatments showed efficacy in trials with fish infected with the parasite and significantly reduced infections of naive fish co-habited with infected fish. Thus, they may have potential as a prophylactic treatment at times of high infection. Three in-feed compounds significantly reduced trophont numbers when administered either for a period of 10 days before infection or for 10 days once infected.

Experiments in Finland to control white spot disease at fish farms

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Field experiments were conducted at three fish farms in Finland in summer 2001. Chemicals were selected according to the preliminary experiments done in 2000. Farm A takes water from a river and the tanks ($6x50m^2$) were cleaned once per week. Farm B takes water from a shallow lake and tanks ($6x50\ m^2$) were not cleaned during the experiment. Farm C takes water straight from a near locating oligotrophic lake and experiments were done in 4 tanks. All treatments (=chemicals) were replicated in three (Farms A and B) or two tanks (Farm C). All experiments were started when natural *I. multifiliis* infection was found in the tanks. Six baths of 5 minutes (except Sodiumpercarbonate 20 min) were used every 2^{nd} or 3^{rd} day (Table 2).

Response variables were the number of *I. multifiliis* on the skin of fish and the cumulative mortality per tank. Malachite-formalin was the control in all experiments.

At farm A all treatments were effective which was interpreted to be caused, at least partly, by the low parasite exposure in the incoming river water and the good maintenance (cleaning) of the tanks.

At farm B the exposure and the parasite burden in the beginning was higher than at A. In four tanks (of six) using alternative treatments (Chloramine T and Per Aqua, a mixture of acetic acid, peracetic acid and hydrogen peroxide) the infection was controlled in such a way that mortality did not exceed 10%. In these tanks bath treatments were, however, still needed with these chemicals after the six baths in the proper experiment. In two tanks

	Farm A	Farm B	Farm C
Fish species and age	salmon 1+	Salmon 1+	rainbow trout 0+
Fish density	220/m^3	120/m^3	1000/m^3
Malachite-formalin 25ppm	X	X	X
Chloramine-T 14-16ppm	X	X	
PerAqua* 40ppm		X	
PerAqua 20ppm + formalin 100 ppm			X
$KMnO_4$ 4ppm	X		
Sodium percarbonate			X

Table 2. Valtonen et al., control of white spot disease. *Hydrogen peroxide + peracetic acid.

malachite-formalin was needed after the experiment due to high mortality.

Results of the experiments at farm C gave corresponding results to those at farm B.

In conclusion, alternative chemicals can be used to lower the parasite burden. Alternative treatments (although much less effective than malachite formalin) and good cleaning of the tanks are needed to keep the parasite burden at a tolerable level until the immunity against this ciliate has been developed, which takes about three weeks. Alternative treatments are needed to be tested also in earth ponds.

Pyceze, ef ficacy and use

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Pyceze® is a 50% solution of bronopol which has been developed for the treatment and control of saprolegniasis on salmonid eggs and fish. Bronopol is an aliphatic halogenitro compound with a wide spectrum of activity against fungal and bacterial cells. Its target is believed to be thiol containing enzymes such as membrane bound dehydrogenases. It is included as a preservative in a wide range of cosmetic and health care products and has an established human safety profile.

Pyceze is now authorised in Norway and Faroes. It has a Provisional Marketing Authorisation in the UK (July 2001) and trial permits in Chile, Ireland and Denmark. Bronopol has been assessed by the CVMP and been placed in Annex II (no MRL required) of Council Regulation 2377/90 for fish eggs and it is anticipated that this will apply also to fish.

The target dose of Pyceze for eggs is 50mg bronopol/litre (1 ml Pyceze/10 litres) for 30 minutes daily from fertilisation to hatch. For fish the dose is 20 mg bronopol/litre (1 ml Pyceze/25 litres) for 30 minutes for 14 consecutive days

These recommendations may be tailored according to local conditions and hatchery systems (recirculation and flow through) and guidance will be provided.

Pyceze has been trialled in several countries for efficacy and safety (including environmental safety). When used according to the recommendations, Pyceze has been shown to be both safe and efficacious for eggs and fish. GCP(v) trial results on a total of 1.4 million eggs on 12 commercial salmon and trout farms demonstrated that Pyceze significantly reduced the degree of fungal infection in salmonid eggs (P=0.00003). Results from 13 salmon and trout farms involving 26,000 fish showed that Pyceze significantly reduced the degree of fungal infection in salmonid fish (P=0.001) and reduced mortality (P=0.001). Bronopol is bio-degradable and does not bio-accumulate.

Experiments in Finland: Is there life still in fish hatcheries without malachite green - Pyceze® and H_2O_2 compared to malachite green

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Aquaculture has relied on malachite green as a treatment for fungal infection in fish hatcheries for several decades. Now, the use of malachite green is not allowed in EU. Therefore, a substitute chemical has to be found or a method for incubation of eggs, e.g. disinfecting inlet water, has to be developed so that no chemicals are needed in hatcheries. Treatment methods against *Saprolegnia* sp. -infection have to be harmless to eggs, environmentally friendly and safe to use.

Saprolegnia sp. infects primarily dead eggs which always are present after stripping and fertilisation. Therefore, quality of fertilised eggs should be the best available but sometimes this might not be sufficiently obtained and other preventive measures against fungal infections are needed. The chemical has to be capable of stopping the spreading of fungal growth from one egg to another.

We tested if Pyceze® and H_2O_2 are capable of stopping the spread of fungal infection on salmon (*Salmo salar*) and brown trout (*Salmo trutta* m. *lacustris*) individually fertilised eggs. Malachite green was used as a control. 10 salmon and 11 brown trout pairs were used. The eggs of one pair were divided into a set of 120 eggs in 5 replicates for every treatment group. Concentrations of Pyceze®, H_2O_2 and malachite green were 100 mg/l, 500 µl/l and 4 mg/l respectively. The eggs were given a bath treatment 3 times a week and for 30 min period until hatching. The spread of fungal growth was analysed by digital photographing.

Survival from fertilisation to the time of hatching was similar in all of the three treatment groups. However, in H_2O_2 treatment group dead eggs infected with *Saprolegnia* sp. related to total number of dead looking eggs was significantly higher than that in Pyceze® or malachite green treatment groups. The number of *Saprolegnia* sp. infected dead eggs increased in all the treatment groups from fertilisation to hatching.

This result was also tested in a field study, 12 l of fertilised rainbow trout eggs in a 90 l bucket. The result in the field study was similar to the experiment of individually fertilised eggs: effect of Pyceze® was similar to malachite green but H_2O_2 could not stop the spreading of fungal growth.

In addition we observed Pyceze® to react with inlet water (=lake water). In the Pyceze® treatment groups we saw that dark brown precipitate was formed during incubation. The precipitation did not cause any damage to eggs but it might cause problems in flow through hatching system e.g. by plugging the water current. The composition of the precipitant is not known.

We conclude that a fish hatchery can function without malachite green. But in the future there is a greater demand for decision making at the farm level - to find out a method suitable just for a hatchery of your own.

Discussion

The general discussion focused on two topics: The legal aspects of the usage of malachite green in the EU countries and on the possibilities of the substitution of malachite green. Several participants expressed the hope of malachite green still being allowed to be used for the treatment of at least eggs and small fry. The official situation is, however, that nobody has applied for the maximum residue limit, MRL, for malachite green from the EU commission. Malachite green is not banned by the commission, but strictly it is not accepted. On the other hand it would be practically impossible that malachite green could pass all the safety criteria for fish, humans and environment. MRL is a part of these criteria and a drug must pass all these evaluations in order to be authorised as a drug in the EU. Same applies for all chemicals to be used in the treatment of fish. Even disinfectants are regarded as drugs, when they are added into the tanks or egg incubators with live fish or eggs. Hydrogen peroxide is, however, one exception of this. It was pointed out that in the future we probably have to forget cheap treatments of fish, because the procedure of passing all the criteria needed for a drug to be authorised will inevitably cost a lot.

The dosage of the mixture of acetic acid, peracetic acid and hydrogen peroxide was emphasised to depend on the quality of the water. The dosage should be especially adjusted according to the organic content of the fish farm water. The technology of using ultraviolet light, ozone and a catalyst together in disinfection of the water was mentioned and it was asked of the experiences or research results of such treatment. In Finland, there are some preliminary but promising results on the usage of UV-light, ozone and hydrogen peroxide for the prevention of saprolegniosis in eggs.