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Treatment of ichthyophthiriasis after malachite green. II. Earth ponds at salmonid farms

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ABSTRACT: We tested formalin, chloramine-T–formalin and Desirox™–formalin, for use against white spot disease (ichthyophthiriasis) caused by *Ichthyophthirius multifiliis* at 3 salmonid farms (*Salmo salar* and *S. trutta* smolt reared in earth ponds). *I. multifiliis* disappeared from most individuals 4 to 5 wk after the first treatment (and after the first *I. multifiliis* were found) with all chemicals, indicating that combinations of these chemicals, and even formalin alone, can be used to lower the parasite burden in earth ponds to such a level that no mortality occurs. This was the case when the fish were treated frequently at the beginning of the infection. Treatment can be stopped once the fish have achieved immunity to ichthyophthiriasis. The developing immunity was also revealed by the distribution of ciliates in the course of the disease. At the beginning of the infection *I. multifiliis* individuals were randomly distributed among the fish, but after 2 to 3 wk, when all the fish were infected, ciliates had increased in numbers and were aggregated in such a way that some fish carried quite heavy burdens. However, over 60% of the fish were free of the parasites after 4 to 5 wk, and had few or no ciliates, meaning that the distribution was even more aggregated. Sea trout had fewer parasites than salmon, and they also recovered from infection earlier even though the treatments and ponds were similar, indicating variation in resistance to *I. multifiliis* between fish species. It was also evident that the chemicals and their concentrations must be planned carefully to suit the conditions at each farm.

KEY WORDS: *Ichthyophthirius multifiliis* · *Salmo salar* · *Salmo trutta* · Fish farming · Immunity · Alternative chemicals

INTRODUCTION

White spot disease is caused by *Ichthyophthirius multifiliis*. Due to the multi-stage life cycle of this organism, infection needs to be prevented by means of repeated baths, for which purpose malachite green together with formalin has for many years been the most effective composition (see Leteux & Meyer 1972, Rintamäki-Kinnunen & Valtonen 1997). Mortality due to white spot disease and methods of preventing the disease in fish reared in concrete tanks since the prohibition of malachite green by an Act of the European Council (valid in Finland from 1 October 2001 onwards) were discussed in our previous paper (Rintamäki-Kinnunen et al. 2005). White spot disease is, nevertheless, especially harmful to fish reared in earth ponds, from which the cysts of the parasites cannot be removed (Valtonen & Keränen 1981, Matthews 1994, Dickerson & Dawe 1995) and it caused the death of more than 80000 one yr old salmon *Salmo salar* L. in earth ponds at a smolt-producing fish farm in northern Finland in the late 1970s (Valtonen & Keränen 1981). After this the disease was treated with malachite green and formalin (Rintamäki-Kinnunen & Valtonen 1997), and no further high mortality from this cause was reported from any Finnish fish farms.

Experiments conducted in concrete tanks in 2001 and 2002 showed that several alternative preventive chemicals or their combinations can be used successfully to lower the parasite burden to such a level that no high mortality occurs, but that it is important that this should be done during the first 3 wk of the infection so that there is time for the fish to develop immunity against these ciliates. Treatment can then be

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reduced and stopped in due course (Rintamäki-Kinnunen et al. 2005). The development and maintenance of immunity to white spot disease has also been documented (see Dickerson & Dawe 1995, Buchmann et al. 2001). Fish have been shown to acquire immunity in 4 wk (when the specific antibodies reached their maximum level in serum) after immunisation at a water temperature of 17.5°C (Aaltonen et al. 1994). *Ichthyophthirius multifiliis* is one of the most effective reproducers among the protozoan parasites as 1 fully grown parasite (tomont) can produce 200 to 800 (Dickerson & Dawe 1995) or even as many as 2000 infective stages in a few days, depending on the water temperature (Bauer 1959). The transmission of infective stages is also very effective, especially in dense fish stocks of uniform age at fish farms (see Wilson et al. 2002 on the influence of host age etc. on parasite distribution). One can, therefore, expect to find a change in the distribution of ciliates among fish, from a random pattern at the beginning of the infection to an aggregated distribution once protective acquired immunity has developed. This is because individual fish differ in their capacity to develop antibodies against *I. multifiliis* (I. Jokinen unpubl.).

The aim of this work was to find alternative treatments and strategies for preventing *Ichthyophthirius multifiliis* infections in earth ponds containing salmon and sea trout of age 1+ yr. The conditions for rearing fish in earth ponds are totally different from those in concrete tanks. In Finland, such ponds are usually between 100 and 1000 m^2, but ponds as large as 4000 m^2 are used at some farms. Rearing density and water turnover rate are lower in earth ponds than in concrete tanks, however. We were looking for a chemical or combination of chemicals which would be effective against white spot disease, cost-effective and as harmless as possible for the environment under earth pond conditions. We also studied the distribution of parasite numbers both at an early stage of the infection and after the stage when most of the ciliates should have been eliminated by the immunity acquired by the fish.

MATERIALS AND METHODS

The experiments were performed at 3 fish farms (A and B in Northern Finland and C in Central Finland) in June/July 2002 [the present Farm A was called Farm B in our previous paper, Rintamäki-Kinnunen et al. 2005]. Farms A and B are situated by large rivers which flow into the Bothnian Bay and are associated with nearby hydroelectric power stations, so that their inflow water comes from the river above the station. Farm C is situated near a lake and takes its water from a river. One yr old salmon *Salmo salar* and sea trout *S. trutta* m. *trutta* reared in 14 earth ponds were used in the experiments (Table 1). No replicates were available at Farm A, but 4 ponds were randomly selected from among 6 of equal size at Farm B and 6 ponds were selected from among 30 of similar size and type at Farm C (Table 2). Altogether 290 000 salmon and 70 000 sea trout were transferred to the earth ponds in May.

In our earlier study we found that a combination of chemicals, e.g. chloramine-T–formalin was more effective in preventing *Ichthyophthirius multifiliis* infections in salmon (Age 1+) than any one of the chemicals tested alone, e.g. chloramine-T. These results were used to choose the chemicals to be studied in this experiment, i.e. formalin alone, Desirox–formalin and chloramine-T–formalin (Table 2). Desirox (Finnish Peroxides) is a combination of peracetic acid (13 %), acetic acid (20 %) and hydrogen peroxide (20 %). The treatments were started when the first *I. multifiliis* were found on each farm and stopped after 4 to 5 wk, when the parasites had disappeared (see dates in Table 2). The fish were treated every 2nd day at Farms A and B, totalling 3 to 4 times per week, whereas at farm C treatments were given on 2 consecutive days, followed by 1 d without treatment and another 2 d of treatment. The fish were not treated at weekends at Farm C. At

Table 1. Fish species and earth ponds used in the experiment at Farms A, B and C in 2002

Farm (species)	No. of ponds	Pond size (m^2)	Water flow ($l\ s^{-1}$)	Fish (no. m^{-3})	Fish mean weight (g [SD])
A (salmon)	2	650, 810	19, 27	66, 57	28.0 [14.2]
A (sea trout)	2	750, 810	30, 22	39, 36	46.3 [20.2]
B (salmon)	2	340	20	125	20.5 [12.9]
B (salmon)	2	820	35	143	20.5 [12.9]
C (salmon)	6	100	18	74	42.4 [14.0]

Table 2. Chemicals and doses used to prevent the spread of *Ichthyophthirius multifiliis* in the experiments at Farms A, B and C in 2002. C = chloramine-T, D = Desirox, F = formalin. Doses in ppm are given after the abbreviation, e.g. F25 for formalin 25 ppm. The number of replicates is given in parentheses

Farm (species)	Date	No. of treatments	Doses
A (salmon)	19/06–29/07	13–17	F25, F50
A (sea trout)	19/06–29/07	13–17	F25, F50
B (salmon)	21/06–16/07	13	D10+F25 (2) D10+F50 (2)
C (salmon)	05/06–01/07	14	D8+F123 (3) C8+F123 (3)

Farm B, Desirox and formalin were first mixed in water in a 1000 l container and then sprayed into the pond, while at Farm C the chemicals were administered separately, formalin being given last. The flush treatment with diluting chemicals lasted about 10 h at Farms A and B and 2 h at Farm C. Dead fish were collected and counted daily.

Random samples of 15 fish per pond were collected before the first bath and after every 3rd bath thereafter. All *Ichthyophthirius multifiliis* trophonts in skin mucus scrapings from about 40 % of the area of the left side of a freshly killed fish (40× magnification) were counted, and for Farm A the Mann-Whitney *U* test was used to assess the differences between the various chemicals and ponds used, while for Farms B and C the differences between the chemicals and ponds were assessed using 2-factor nested ANOVA with treatment and pond as fixed factors. The differences between the ponds which received the same treatment were further tested using *a priori* defined contrasts. All the analyses were performed on log-transformed, time-reduced data. This meant that the samples taken at the beginning and at the end of the experiment, when *I. multifiliis* was generally not observed, were ignored in the analyses. Thus, the data used were collected over 3 wk (25 June to 16 July 2002) at Farm A, over 2 wk (26 June to 8 July 2002) at Farm B and over 2.5 wk at Farm C (14 June to 1 July 2002).

The distribution of parasites on the fish was studied by pooling 2 successive samples from the salmon pond treated with formalin 25 ppm (F25) at Farm A. The first time, the fish sampled before the treatments and after 3 baths the samples were pooled. The 2nd pooling consisted of the samples collected on 3 and 9 July, when all the fish were infected, and the 3rd consisted of those collected 16 and 23 July. The aggregation of *Ichthyophthirius multifiliis* trophonts on fish was measured using the variance-to-mean ratio.

RESULTS

Experiments at Farm A

The first *Ichthyophthirius multifiliis* individuals were found in all the ponds on 18 June, with a prevalence of 6.7 % in all cases. The average intensity was 1 ciliate per mucus sample from an infected fish. Formalin treatments (F25 and F50 = formalin 25 ppm and 50 ppm, respectively) were started the next day. After 6 baths and a period of 2 wk starting from the onset of infection, the mean (SD) number of parasites had increased to 67.0 (30.4) and 45.5 (30.7) on salmon receiving the F25 and F50 baths, respectively, and 72.1 (43.9) and 16.0 (11.6) on the sea trout receiving the same doses. Salmon given F25 had significantly more parasites in the time-reduced pooled samples than the F50 salmon (Mann-Whitney $U = 4377.5$, $p = 0.010$, Fig. 1), but no corresponding differences were found in the pooled sea trout samples (Mann-Whitney $U = 1498.5$, $p = 0.11$, Fig. 1). Also, more parasites were found in the salmon than in the sea trout. In both ponds the parasite burden on the sea trout had decreased after 4 wk and 12 baths (mean 2.5 [8.7] and 5.0 [8.8] in F25 and F50, respectively), this was not the case for the salmon, however (mean 125.3 [130.1] and 14.8 [11.5] in F25 and F50, respectively). After 4 wk the sea trout were treated only once and the salmon 5 times. Mortality in

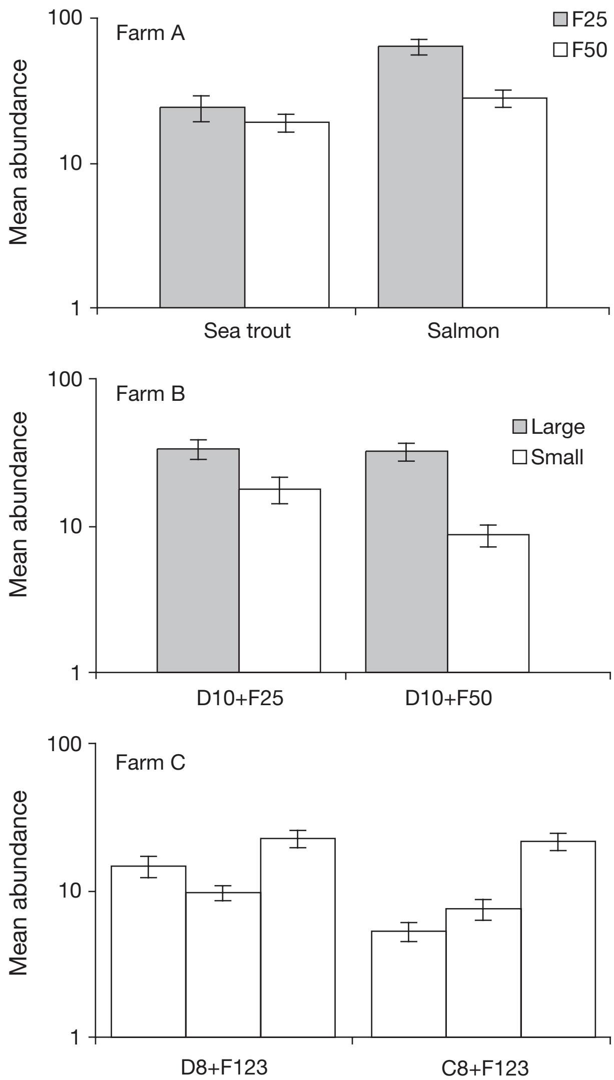

Fig. 1. Mean abundances (±SE) of *Ichthyophthirius multifiliis* in the 4 earth ponds at Farm A, 2 replicates of the 2 treatments at Farm B and 3 replicates of the 2 treatments at Farm C (log-transformed, time-reduced data; see 'Materials and methods'). C = chloramine-T, D = Desirox, F = formalin. Doses in ppm are given after the abbreviation, e.g. F25 = formalin 25 ppm. Large: large pond (820 m^2); Small: small pond (340 m^2)

all 4 ponds during the 5 wk of the experiment was below 1%.

We studied the distribution of parasites on fish by pooling 2 successive samples from the salmon pond treated with F25. Only a few parasites were found on the skin mucus (max. 4) before the treatments and after 3 baths, and only 36.7% of the fish (n = 30) were infected, the variance to mean ratio being 1.92, showing an almost random distribution. During the second period, the maximum number of parasites in a mucus sample from 1 fish was 251 (mean 107.7, SD 68.4) (Fig. 2) and the distribution of the parasites was more aggregated, the variance-to-mean ratio being 43.5. In the third period, 63.3% of the fish had no or fewer than 15 parasites and 10% had over 200 parasites, the variance-to-mean ratio being 192.2, implying very pronounced aggregation (Fig. 2).

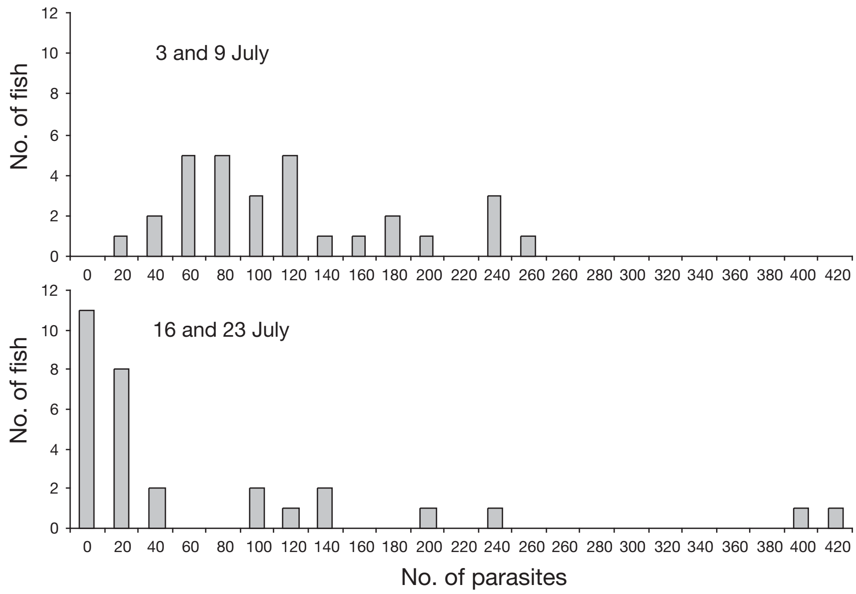

Fig. 2. Distribution of *Ichthyophthirius multifiliis* on fish at Farm A in 2002. Two successive samples from the salmon pond treated with formalin (25 ppm) were pooled

Experiments at Farm B

The first few parasites were found on 19 June and the treatments with Desirox–formalin (D10+F25 and D10+F50 = Desirox 10 ppm and formalin 25 ppm or 50 ppm, respectively) were started 2 d later. The highest numbers of parasites were found after 6 baths given over a period of 2 wk, starting from the first observation of *Ichthyophthirius multifiliis* (mean 38.7, SD 28.6 and 37.0, SD 5.5 in the D10+F25 and D10+F50 ponds, respectively). After 9 baths (3 wk) the number of parasites had decreased in all 4 ponds to mean values of 32.7, SD 6.6 and 16.7, SD 4.1, respectively. No significant differences were found between the 2 treatments, however (Table 3, Fig. 1). There was significant variation among the ponds receiving the same treatment (Table 3, Fig. 1), this being most marked in the D10+F50 treatment (F = 29.941, df 1, 175, $p < 0.001$ and F = 5.114, df 1, 175, $p = 0.025$ in D10+F50 and D10+F25, respectively). After 12 baths in 4 wk, 18.3% of the sample fish (n = 60) had parasites and only 1 to 4 parasites were found per infected fish. Mortality was below 1% in all ponds during the experiment.

Experiments at Farm C

The first *Ichthyophthirius multifiliis* were noted on 4 June and treatments were started on 5 June. During the 5 wk of the experiment the number of parasites remained very low in all the ponds, the means being 2.9, SD 2.7 and 1.7, SD 1.5 on fish in the Desirox-formalin (D8+F123) and chloramine-T–formalin (C8+F123) ponds, respectively after 6 baths within 1.5 wk and 16.7, SD 11.8 and 15.5, SD 11.6, respectively after 9 baths within 2.5 wk. Only a few fish had more than 50 parasites (max 126). The number of parasites was significantly higher in the D8+F123 than in the C8+F123 treatments (Table 3, Fig. 1), but there was also significant variation among the ponds receiving the same treatment (Table 3, Fig. 1), this being greater in the C8+F123 treatment (F = 20.950, df 2,350, $p < 0.001$ and F = 3.350, df 2,350, $p = 0.036$ in C8+F123 and D8+F123, respectively). Mortality was below 1.5% in all the ponds.

Table 3. Effect of treatment and pond within the same treatment on the mean abundance of *Ichthyophthirius multifiliis* (nested ANOVA) at Farms B and C in 2002 (log-transformed, time-reduced data; see ‘Materials and methods’)

Farm	Factor	MS	F	df	p
B	Treatment	0.592	2.666	1	0.104
	Pond (Treatment)	3.890	17.527	2	<0.001
	Error	0.222		175	
C	Treatment	2.408	10.556	1	0.001
	Pond (Treatment)	2.772	12.150	4	<0.001
	Error	0.228		350	

DISCUSSION

In all 14 ponds studied at the 3 fish farms *Ichthyophthirius multifiliis* disappeared 4 to 5 wk after its first appearance and the first treatment, regardless of the chemical or combination of chemicals used. This mirrors the results of experiments in concrete tanks at 2 farms in 2001 and 2002 (see Rintamäki-Kinnunen et al. 2005). We are of the opinion that the disappearance of the parasites was attributable to acquired resistance to *I. multifiliis*, as also demonstrated by Hines & Spira (1974) and later by Wahli & Meier (1985) and reviewed by Buchmann et al. (2001). The developing immunity was indicated here by the distribution of ciliates in the course of the infection, as shown at Farm A. At the beginning of the infection *I. multifiliis* individuals were randomly distributed among the fish, but by the time all the fish were infected, i.e. after 2 to 3 wk, the ciliates had increased in number and were aggregated in such a way that some fish were carrying quite heavy burdens. After 4 to 5 wk, however, over 60 % of the fish were free of the parasites, having no or only few ciliates, and the distribution was even more aggregated. Heterogeneities in host–parasite interactions are normally generated by variation between individuals in their exposure to infective stages and their susceptibility once an infectious agent has been encountered (see Wilson et al. 2002). In the present case all the fish were exposed to *I. multifiliis* at an early stage of infection and were also infected, but the development of acquired immunity within 4 wk was shown by the rejection of the ciliates by most fish and a change in distribution towards more pronounced aggregation. This supports the key role of the development of immunity in 'cleaning' the fish. Since no naïve fish were introduced into the ponds before, during or after the experiment, it is most likely that the tail of the distribution (the proportion of fish with a heavy ciliate burden) at the stage of advanced infection reflects genetic differences, implying that those fish were in poorer condition, for example, and were weaker at developing resistance to *I. multifiliis*. Indeed, 20 % of the fish had over 200 parasites in their mucus sample while another 20 % had freed themselves entirely of the parasites 4 wk after the beginning of the infection.

Variation in resistance to *Ichthyophthirius multifiliis* between fish species and between populations or strains of a single species has been observed previously. Butcher (1947) found that rainbow trout *Oncorhynchus mykiss* were more susceptible to *I. multifiliis* infection than were brown trout *Salmo trutta*, while later, Clayton & Price (1992) and Price & Clayton (1999) reported both interspecific and intraspecific variation in resistance to *I. multifiliis* among poeciliid and goodeid fish and in the common carp *Cyprinus carpio*, respectively. The sea trout in the present study had fewer parasites than the salmon and also recovered from ciliate infection earlier than the salmon, even though the treatments and ponds were similar. Since we were not allowed to use untreated controls at any of the farms, we do not know whether the sea trout would have managed to free themselves of the parasites even if they had not been given any treatment. However, sea trout reared in 8 small earth ponds (250 m^2) at one further farm in Northern Finland were treated with formalin (25 ppm) only 1 to 3 times for an *I. multifiliis* infection in 2002, and no appreciable parasite burden or mortality could be found (P. Rintamäki-Kinnunen unpubl.).

The size of an earth pond and its water flow rate would seem to have an effect on the severity of *Ichthyophthirius multifiliis* infection. It was found in an earlier study that the water turnover rate was important in controlling white spot disease, so that an increase in the turnover rate from 0.5 times h^{-1} to 2.5 h^{-1} reduced the mortality of channel catfish *Ictalurus punctatus* infected with *I. multifiliis* from nearly 100 % to less than 10 % (Bodensteiner et al. 2000). The present parasite burden was lowest at Farm C, where the smallest ponds were used (100 m^2) with a turnover rate of 0.5 times h^{-1} vs. about 0.1 h^{-1} at Farms A and B. Also, at Farm B fewer parasites were found on the salmon in the smaller ponds than in the larger ponds (340 vs. 820 m^2).

In a situation where the use of highly effective therapeutic chemicals such as malachite green is forbidden and where no other equally effective chemicals are available, we found several treatments to be of some use in controlling ichthyophthiriasis. Formalin is one of the most widely used therapeutic agents in fish culture and has been used to treat ichthyophthiriasis for many years (e.g. Piper et al. 1982), but opinions differ on its effectiveness with white spot disease (Prost & Studnicka 1972, Valtonen & Keränen 1981, Wahli et al. 1993, Tieman & Goodwin 2001). The present higher dose of formalin (50 ppm) was more effective in controlling *Ichthyophthirius multifiliis* infections in salmon at Farm A than formalin 25 ppm, but no differences were found between the 2 doses of formalin in combination with Desirox (D10+F25 and D10+F50) at Farm B, although the difference was clear in the smaller ponds there (see Fig. 1: higher dose more effective; results not given). No differences in the degree of gill hyperplasia at the end of the experiment were found between the chemicals used at Farms A and B (P. Koski et al. unpubl.).

It was also noted that there were no differences between formalin (F50) and Desirox-formalin (D10+F50) at Farms A and B when used for treating salmon in earth ponds of similar sizes, in spite of the fact that Desirox–formalin was the best of the 3 combinations of chemicals for treating ichthyophthiriasis in fish raised in concrete tanks (the other combinations tested were chloramine-T–formalin and hydrogen peroxide-formalin) (Rintamäki-Kinnunen et al. 2005). The situation in earth ponds is evidently not so clear, for Desirox–formalin was actually less effective than chloramine-T–formalin at Farm C. It is possible that earth ponds may have a high organic load and that this may mean that Desirox–formalin is not as effective an alternative as in concrete tanks. On the other hand, differences were observed between the ponds in which the same treatment was applied, as was also the case for concrete tanks. This was found in particular when chloramine-T was used in combination with formalin at Farm C, where a substantially higher burden of parasites was found in 1 of the 3 chloramine-T–formalin ponds. As stated earlier (Rintamäki-Kinnunen et al. 2005) more experimental work would be needed to be sure of the efficiency of chloramine-T–formalin, especially in earth ponds. The higher costs of chloramine-T relative to formalin is also a significant point when treating earth ponds, which may be quite large. In the end, formalin alone may prove to be a good choice for treating white spot disease in earth ponds.

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