Vol. 64: 69–76, 2005 | DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org | Published April 6

Treatment of ichthyophthiriasis after malachite green. I. Concrete tanks at salmonid farms

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ABSTRACT: Since the use of malachite green was banned in many European countries, new alternative treatments have been tested to prevent white spot disease caused by *Ichthyophthirius multifiliis*. We tested formalin, potassium permanganate ($KMnO_4$), chloramine-T, hydrogen peroxide (H_2O_2) and Per Aqua or Desirox alone or in combinations of 2 chemicals, one of which was always formalin, in 50 m^2 concrete tanks at 2 farms producing salmon *Salmo salar* smolt in 2001 and 2002. Both Per Aqua and Desirox are combinations of peracetic acid, acetic acid and hydrogen peroxide. The alternative chemicals or their combinations can be used successfully to lower the parasite burden to such a level that no high mortality occurs during the first 4 wk after the start of an infection. This period of time allows the fish to develop immunity against these ciliates, and treatments can be reduced and stopped in due course. *I. multifiliis* decreased in number 3 to 4 wk after the beginning of the infection in all the treatments. Large differences in parasite burden and mortality occurred among the replicates in all except the Desirox-formalin tanks, which means that they are not as reliable as the malachite green-formalin used previously. It was also evident that the chemicals and their concentrations must be planned carefully to suit the conditions on each farm.

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

INTRODUCTION

White spot disease (ichthyophthiriasis), caused by *Ichthyophthirius multifiliis*, occurs in both wild and cultured fish. Without any preventive treatment it may cause high mortality among both juvenile and adult fish (Elser 1955, Valtonen & Keränen 1981, Majeed et al. 1984, Wurtsbaugh & Tapia 1988, Traxler et al. 1998). Many chemicals have been tested for preventing the disease, e.g. malachite green, malachite green in combination with salt or formalin, formalin, chloramine-T, potassium permanganate and copper sulphate (Johnson 1961, Ljunberg 1963, Prost & Studnicka 1972, Cross & Hursey 1973, Schachte 1974, Straus 1993, Schlenk et al. 1998, Straus & Griffin 2002). Malachite green with formalin has been found to be the best alternative (Bauer et al. 1969, Wahli et al. 1993, Rintamäki-Kinnunen & Valtonen 1997, Tieman & Goodwin 2001).

White spot disease was reported at Finnish fish farms for the first time in the mid-1970s, and it killed over 80 000 salmon *Salmo salar* L. at a smolt-producing farm in 1978 (Valtonen & Keränen 1981). About 95% of the salmon smolt in Finland from the 1950s onwards have been produced at fish farms and stocked due to the building of hydroelectric power stations on the rivers. This has meant the production of over 5 million salmon per year from the mid-1990s onwards. Since the late 1970s white spot disease has been treated with malachite green and formalin, and no serious mortality has been caused by *Ichthyophthirius multifiliis* outbreaks during the last 2 decades (see Rintamäki-Kinnunen & Valtonen 1997). The use of malachite green was nevertheless prohibited from 1 January 1998 onwards by an Act of the European Council, on account of the possible toxic effects it may have on fish consumers (see Meyer & Jorgenson 1983), although its use in Finland was permitted until 1 October 2001 by the National

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Agency for Medicines in order to facilitate research into alternative preventive methods.

New treatments for white spot disease have been tested in many countries. New chemicals such as toltrazuril, sodium percarbonate and garlic extract have been considered in Spain and Denmark, for example, but they were not effective against *Ichthyophthirius multifiliis* (Tojo et al. 1994, Buchmann et al. 2003). Oral chemotherapeutics have been tested in Scotland (Shinn et al. 2003) and in Spain (Rodriguez & Fernandez 2001, Luzardo-Álvarez et al. 2003), and some have been found to reduce the number of *I. multifiliis* trophonts and may in future be real alternative treatments for white spot disease. Some 'old' chemicals which have been used for decades in fish culture have also been tested and found to be effective against *I. multifiliis*, e.g. potassium permanganate for controlling *I. multifiliis* epizootics in channel catfish (Straus & Griffin 2002).

We studied alternative preventive methods during the summer from 2000 to 2002. Preliminary laboratory tests were performed in 2000 to trace suitable alternatives for further experimentation, leading to the choice of certain chemicals for testing under real fish farming conditions in 2001. The results obtained in both years were then used to select chemicals for further tests in 2002, also under fish farming conditions. The aim was to find an alternative chemical or combination of chemicals which would be as environmentally friendly as possible and at the same time effective against *I. multifiliis*. We report here on the results of experiments carried out in 50 m^2 concrete tanks in 2001 and 2002. The results of corresponding experiments in earth ponds will be given in a subsequent paper.

MATERIALS AND METHODS

The field tests were performed at 2 fish farms (A and B) in northern Finland in July/August 2001 and 2002. Farm A is situated between 2 lakes and takes its inflow water from a small eutrophic lake. Farm B is situated by a large river which flows into the Bothnian Bay. It is associated with a nearby hydroelectric power station and its inflow water comes from the river above the station. One yr old salmon *Salmo salar* were used in the experiments. The fish were reared in 9 randomly chosen concrete tanks out of the 64 tanks at Farm A in 2001 and 2002 and a further 9 out of the 12 concrete tanks at Farm B in 2001 (Table 1). Altogether, 153 000 and 45 000 salmon in 2001 and 2002, respectively, were transferred into these tanks in late May at Farm B and in late June/early July at Farm A. The concrete tanks were cleaned out by brushing every second week during the summer at Farm B, but no brushing of the tanks took place at Farm A.

Formalin, potassium permanganate ($KMnO_4$), chloramine-T, hydrogen peroxide (H_2O_2), Per Aqua (Nordic Brenntag) and Desirox (Finnish Peroxides) were used for treatments, either alone or in combinations of 2 chemicals, one of which was always formalin (Table 2). Both Per Aqua and Desirox are combinations of peracetic acid (13%), acetic acid (20%) and hydrogen peroxide (20%). We changed Per Aqua for a similar but cheaper domestic product, Desirox, in 2002. Malachite green–formalin was used as a control in 2001 but permission could no longer be obtained to use it in 2002. Similarly the permit for the experiments did not allow us to use untreated controls.

The experiments were started in 2001, when a natural *Ichthyophthirius multifiliis* infection had been noted in the tanks and its level was as even as possible among them (Fig. 1). The number of treatments per

Table 1. Tanks used in the experiments at Farms A and B in 2001 and at Farm A in 2002. Nine tanks were used for all experiments

Farm	Tank size (m^2)	Water flow ($l\ s^{-1}$)	Fish (m^{-3})	Mean weight of fish (g)
2001				
A	50	9	120	12.1
B	50	11	220	40.0
2002				
A	50	9	83	14.7

Table 2. Chemicals and doses used for the prevention of *Ichthyophthirius multifiliis* in the experiments at Farms A and B in 2001 and at Farm A in 2002. C = chloramine-T, P = potassium permanganate, PA = Per Aqua, MF = malachite green-formalin, D = Desirox, F = formalin, H = hydrogen peroxide. Dose is given as ppm after the letter, e.g. C16 for chloramine-T 16 ppm. The number of replicates is given in parentheses

Farm (Date of Expt, dd/mm)	Chemical and dose	No. of treatments per tank
2001		
A (20/07–13/08)	C14 (3)	7 to 10
	PA40 (3)	
	MF[a] (3)	
B (23/07–06/08)	C16 (3)	6
	P4 (3)	
	MF[a] (3)	
2002		
A (08/07–09/08)	C10+F100 (3)	16
	D10+F100 (3)	
	H100+F100 (3)	

[a]3.7 g malachite green was mixed to 1 l formalin and from this mixture 225 ppm was used

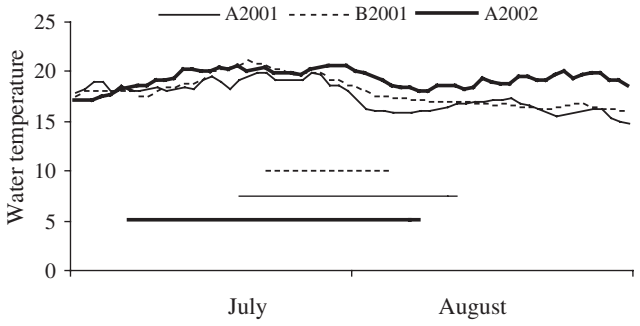

Fig. 1. Water temperatures (°C) and time and duration of experiments (straight lines) at Farms A and B in 2001 and at Farm A in 2002

tank was 7 to 10 at Farm A and 6 at Farm B. The chemicals tested at Farm A are given in Fig. 2, including those given in cases of increased mortality. A change in the test chemical to malachite green–formalin or formalin was advised in cases of increased mortality in the permit for the experiment given by Lab-Animal Care and Use Committee of the University of Jyväskylä. In 2002, the treatments were started at Farm A when the first parasites were found in any of the experimental tanks and discontinued when most of the parasites had disappeared (see dates in Table 2). All the fish died after the 6th bath in 1 hydrogen peroxide–formalin tank and 1 Desirox–formalin tank, evidently not on account of the *I. multifiliis* infection but because of the wrong doses of the chemical. The results for these tanks were excluded from analyses. In addition, to achieve a balanced design for the statistical comparisons, 1 randomly chosen chloramine-T tank was also omitted.

The fish were treated 3 times a week, i.e. every 2nd day, in both years, but not during the weekends. The water level in the tanks was lowered from ca. 1 m to 30 cm (water volume from 50 to 15 m^3) before the treatment. The chemicals were spread in the tanks separately, with formalin always given after the other chemical in the combination cases, except in the case of malachite green–formalin (see Table 2). The flow of water into the tank was stopped for 5 min at Farm A but not at Farm B. Treatment with the diluting chemicals lasted ca. 2 h at both farms. Dead fish were collected from the tanks and counted daily.

Random samples of 15 fish per tank were collected before the first bath and later after every 3rd bath. Smaller samples of 4 to 11 fish per tank were also collected at Farm A in 2001 in addition to the main samples. Skin mucus scrapings were taken from ca. 40% of the area of the left side of a freshly killed fish and all *Ichthyophthirius multifiliis* trophonts were counted (40× magnification). Differences between the chemicals and between the tanks within each treatment were assessed using 2-factor nested ANOVA. In the case of a significant difference between the chemical treatments, multiple comparisons were made using Tukey's test. For differences among tanks within one treatment, custom hypotheses were specified and further compared using *a priori*-defined contrasts. Analyses were performed on log-transformed, time-reduced data, which meant that the samples taken at the beginning and end of the experiment, which mainly had no *I. multifiliis* observations, were ignored in the analyses. Thus the data used in the analyses were collected over 2.5 wk (23 July to 8 August 2001) at Farm A (see Fig. 2) and over 1 wk (23 to 27 July 2001) at Farm B. In 2002, the first 2 samples (8 and 12 July) and the last one (14 August) taken at Farm A were eliminated and data collected over 3 wk (19 July to 9 August) were used in the analyses.

RESULTS

Experiments at Farm A

The first *Ichthyophthirius multifiliis* were found in 6 of the 9 tanks on 16 July 2001, and the experiments with chloramine-T, Per Aqua and malachite green–formalin were started on 20 July 2001 (Table 2). The water temperature was between 15.7 and 20.0°C during the experiment (Fig. 1). Parasite numbers at the beginning of the experiment averaged 4.1 (SD 3.1) per fish (range 0 to 15), and after 3 baths the average was already 48.2 (SD 35.4) and the range 5 to 179. There

Table 3. Effect of treatment and tank within each treatment on the mean abundance of *Ichthyophthirius multifiliis* (nested ANOVA) at Farms A and B in 2001 and and at Farm A in 2002 (log-transformed, time-reduced data; see 'Materials and methods')

Farm	Factor	MS	*F*	df	p
2001					
A	Treatment	40.799	116.642	2	<0.001
	Tank (Treatment)	1.747	4.994	6	<0.001
	Error	0.350		558	
B	Treatment	0.024	0.093	2	0.912
	Tank (Treatment)	0.253	2.164	6	0.047
	Error	0.117		261	
2002					
A	Treatment	7.834	18.655	2	<0.001
	Tank (Treatment)	2.654	6.321	3	<0.001
	Error	0.420		353	

were significant differences among the treatments (Fig. 2, Table 3), the number of parasites being significantly higher in the chloramine-T and Per Aqua treatments than in the malachite green–formalin tanks (Tukey's test for pairwise differences, $p < 0.001$), but the differences between the chloramine-T and Per Aqua treatments were not significant (Tukey's test, $p = 0.728$). There was also significant variation among the tanks within each of the treatments, however (Fig. 3, Table 3, $F > 3.682$, df = 2, 558, $p < 0.027$ for all treatments).

In 2 chloramine-T and 2 Per Aqua tanks, malachite green–formalin or formalin was used after 4 to 6 treat-

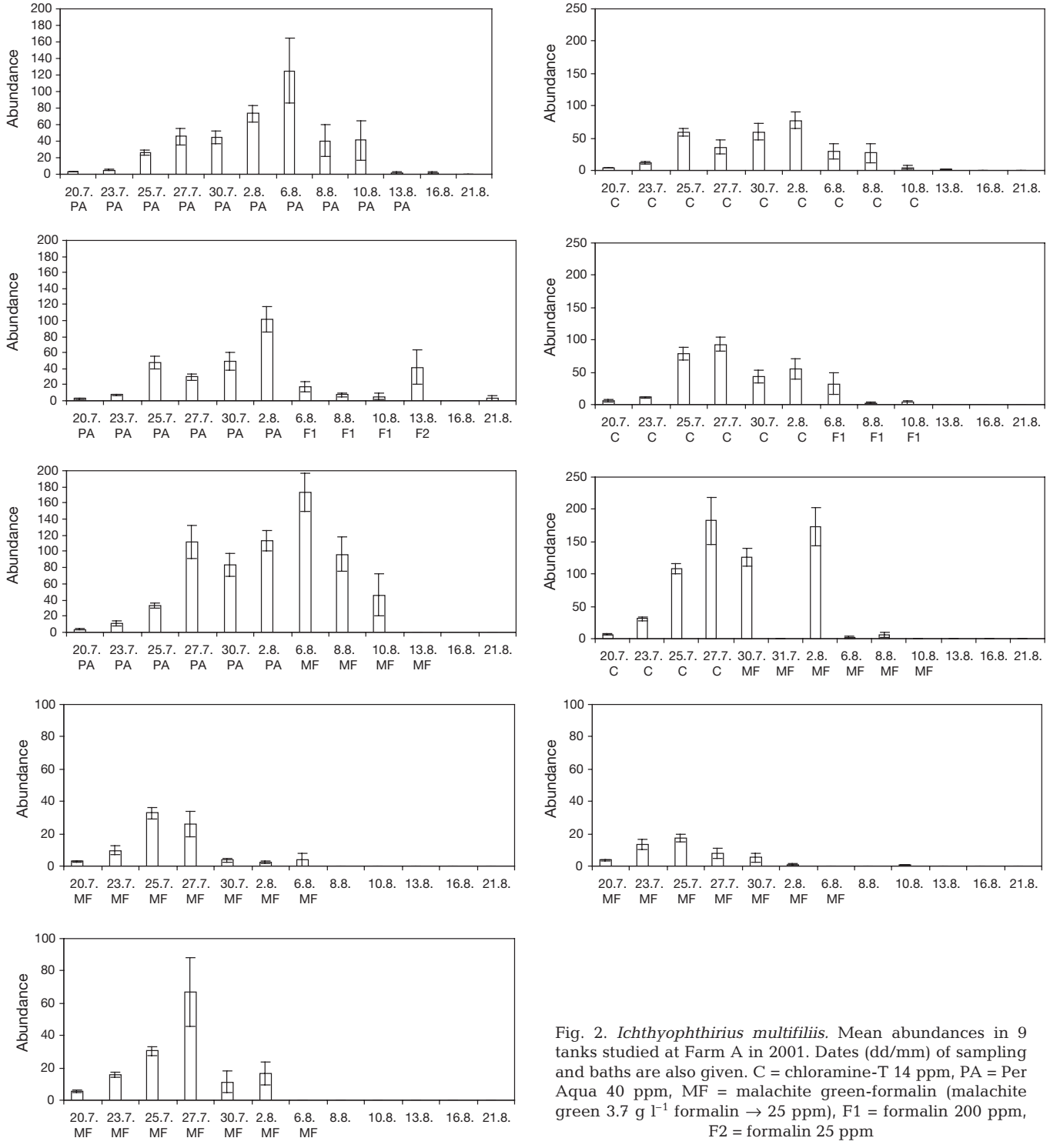

Fig. 2. *Ichthyophthirius multifiliis.* Mean abundances in 9 tanks studied at Farm A in 2001. Dates (dd/mm) of sampling and baths are also given. C = chloramine-T 14 ppm, PA = Per Aqua 40 ppm, MF = malachite green-formalin (malachite green 3.7 g l^{-1} formalin → 25 ppm), F1 = formalin 200 ppm, F2 = formalin 25 ppm

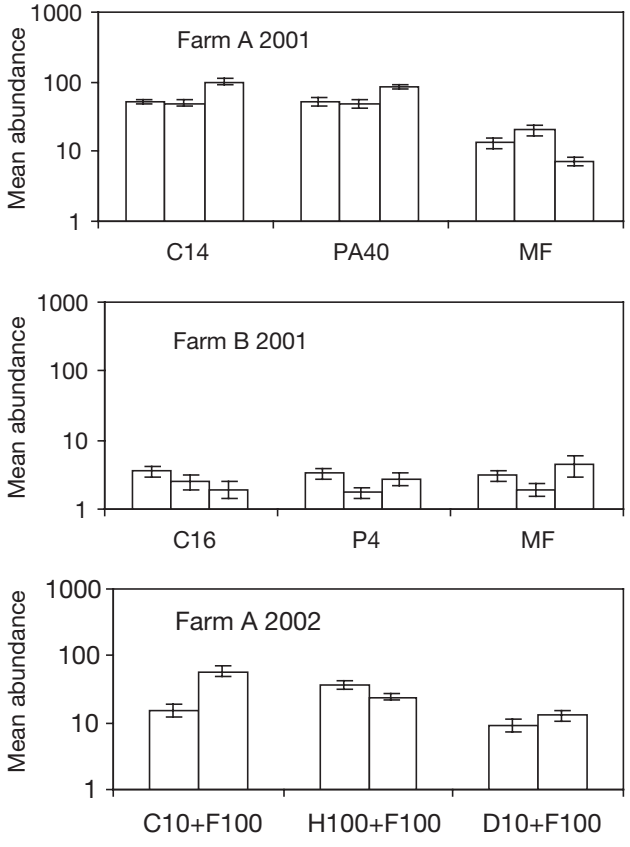

Fig. 3. *Ichthyophthirius multifiliis.* Mean abundances in the 3 replicates of the 3 treatments at Farms A and B in 2001 and in the 2 replicates at Farm A in 2002 (log-transformed, time-reduced data; see 'Materials and methods'). C = chloramine-T, P = potassium permanganate, MF = malachite green–formalin (malachite green 3.7 g l^{-1} formalin → 25 ppm), PA = Per Aqua, D = Desirox, F = formalin, H = hydrogen peroxide. Dose is given as ppm after the letter, e.g. C16 for chloramine-T 16 ppm

ments because of the high number of parasites or increased mortality (Fig. 2). The highest mortality was found in 1 of the 3 chloramine-T tanks, where ca. 30% of the fish died during the 3 wk period. In the other 2 chloramine-T tanks, mortality at the same time was ca. 3%. Mortality in the Per Aqua tanks was 4 to 11%, and that in the malachite green–formalin tanks only 0.5%.

Summer 2002 was warmer than that of 2001 (Fig. 1), and the salmon were found to be infected with *Ichthyophthirius multifiliis* at the beginning of July, 1 wk after they had been transferred to the tanks. The treatments with chloramine-T–formalin, hydrogen peroxide–formalin and Desirox–formalin were started on 8 July, when parasites were found in 5 of the 9 tanks (Table 2).

Significant differences in parasite burden occurred between the chemical treatments and also between the tanks within the treatments (Fig. 3, Table 3). The number of parasites was significantly higher in the hydrogen peroxide–formalin and chloramine-T–formalin treatments than in the Desirox–formalin tanks (Tukey's test, $p < 0.001$ in both cases), but the differences between the hydrogen peroxide–formalin and chloramine-T–formalin treatments were not significant (Tukey's test, $p = 0.733$). Differences were observed between the tanks within the same treatment only for chloramine-T ($F = 15.556$, df = 1, 353, $p < 0.001$). Mortality was below 2% in the 2 Desirox–formalin tanks during the experiment, 6 and 13% (only 2 tanks, see 'Materials and methods') in the hydrogen peroxide–formalin tanks and highest in 1 of the 3 chloramine-T–formalin tanks (2, 8 and 37%).

Experiments at Farm B

The first *Ichthyophthirius multifiliis* were recorded in 5 of the 9 tanks on 9 July 2001, and the experiment was started on 23 July, when the fish in all the tanks were infected (Table 2). Water temperature was 21.2°C at the beginning of the experiment and 17.6°C at the end (Fig. 1). The overall number of *I. multifiliis* was low at the farm, e.g. mean number of parasites after 3 baths was 2.2 (SD 3.9) per fish (range 0 to 39). There were no differences among chemical treatments, although there was obvious variation among the tanks within the chloramine-T treatment (Fig. 3, Table 3, test for differences among tanks within chloramine-T, $F = 3.363$, df = 2, 261, $p = 0.036$). Mortality was low during the 2 wk when the baths were given. The majority of dead fish were found in the tanks treated with potassium permanganate (mortality 0.5 vs. 0.1% in the other tanks).

DISCUSSION

Since the use of malachite green was prohibited in many European countries, new alternative treatments have been tested for preventing white spot disease caused by *Ichthyophthirius multifiliis*. The present results show that the alternative chemicals or combinations can be used to lower the parasite burden in concrete tanks to such a level that no high mortality occurs during the first 4 wk after the start of infection. This time allows the fish to develop immunity against these ciliates, and treatments can be reduced and stopped in due course. *I. multifiliis* decreased in numbers within 3 to 4 wk of the beginning of the infection and the treatments. Another of the main findings is that large differences in parasite burden and mortality occurred among the replicates within each treatment, except for Desirox–formalin tanks at Farm A in 2002. This means

that the alternative treatments are not as reliable as the malachite green–formalin used previously.

Combinations of 2 chemicals, especially Desirox–formalin, were found to be more efficient for treating *Ichthyophthirius multifiliis* than one chemical alone. Higher numbers of parasites were found in the tanks treated with chloramine-T and Per Aqua in 2001 than in those treated with chloramine-T–formalin and Desirox–formalin in 2002. The inefficiency of chloramine-T and Per Aqua alone is also seen in the fact that in only 1 of the 3 replicate tanks for each treatment was the original chemical used throughout the experiment, while the others received malachite green–formalin or formalin alone in the latter half of the experiment due to increased mortality.

The early summer of 2002 was very warm and *Ichthyophthirius multifiliis* infections broke out 1 to 2 wk earlier than during the 2 previous summers, but parasite burdens were lower than in 2001. This may be attributed to the better efficiency of the combination of 2 chemicals (one always being formalin). Another reason may be quicker activation of immunity to *I. multifiliis* ciliates at higher temperatures. Developing immunity to ichthyophthiriasis has been reported many times, as seen in the review article by Dickerson & Dawe (1995). Fish surviving *I. multifiliis* outbreaks become resistant to reinfection; Hines & Spira (1974), for example, found that carp *Cyprinus carpio* achieved immunity to ichthyophthiriasis in 3 wk. A specific antibody response was recorded in the roach *Rutilus rutilus* 4 wk after immunisation at Finnish summer temperatures of 17.5°C (Aaltonen et al. 1994). Similarly, the parasites in all our experiments started to disappear 3 or 4 wk after the beginning of the infection, i.e. after the time when the first *I. multifiliis* were found.

A very low parasite burden was found at Farm B compared with that at Farm A. This is important, especially as the rearing density of the fish was double that used at Farm A and the fish were bigger. Even then, *Ichthyophthirius multifiliis* disappeared after 6 baths given over 2 wk, and no differences were found between the chemicals, chloramine-T, potassium permanganate and malachite green–formalin. One reason may be the brushing of the tanks every 2nd wk during the summer. We would maintain, however, that the main reason should be sought in the water intake, as the water at that farm comes straight from a large river. It has been shown that roach may even have 100% *I. multifiliis* infection in July in polluted eutrophic lakes (Valtonen & Koskivaara 1994). The large river from which Farm B takes its water has sparse populations of cyprinids compared with eutrophic lakes, while Farm A takes its water from a small eutrophic lake with a high cyprinid density. Farm A will thus be more likely to receive *I. multifiliis* theronts from wild fish in the inflow water. The flow rate of the water is also important for the control of white spot disease, and this too is slightly higher at Farm B. Bodensteiner et al. (2000) found that when the flow rate in raceways was increased from 5 to 15 l min^{-1}, the mortality of channel catfish *Ictalurus punctatus* due to white spot disease was only half of what it was at the lower flow rate.

The fish farming industry will need to devote more attention and resources to the treatment of white spot disease in the future. It is also evident that the chemicals and their concentrations must be planned carefully to suit the conditions at each farm. We made certain observations in our experiments which may influence the choice of chemical or chemical combination when planning treatments. The toxicity of hydrogen peroxide increases with water temperature (Johnson et al. 1993, Bruno & Raynard 1994, Rach et al. 1997). Water temperatures in the present study were between 16 and 21°C when we used hydrogen peroxide in combination with formalin. We also used Per Aqua and Desirox, which both contain 20% hydrogen peroxide. The hydrogen peroxide doses we used followed the recommended treatment range from 50 to 250 ppm for exposures up to 60 min (Rach et al. 1997). In the Per Aqua (40 ppm) and hydrogen peroxide–formalin (both 100 ppm) tanks, mortality increased to a moderate level (4 to 13%) during the experiments at Farm A in both years, whereas when Desirox (10 ppm) was used in combination with formalin (100 ppm) mortality was below 2% and the parasite burden was the lowest of the 3 treatments used at Farm A in 2002. The degree of gill hyperplasia was also lower in the Desirox–formalin-treated fish than in chloramine-T–formalin or hydrogen peroxide–formalin treated ones (P. Koski et al. unpubl.). Although the hydrogen peroxide–formalin tanks were cleaner than those with other treatments in 2002, the lower doses of chemicals containing hydrogen peroxide seem to be safer for the fish.

Differences between tanks within the same treatment were observed when chloramine-T was used alone or in combination with formalin at Farm A, and also at Farm B, where parasite burdens were low. In 1 of the 3 chloramine-T-treated tanks, high mortality was found (Farm A) in both years. Acidity is known to be the most important factor affecting chloramine-T toxicity, which is about 6 times greater at pH 6.5 than at pH 9.5 (Bills et al. 1988). The pH at Farm A in July 2002 was 6.7 (R. Kannel pers. comm.). Although the chloramine-T concentration used in 2002 (10 ppm) was in accordance with the recommended dose (10 ppm in soft water, pH 7) (see Cross & Hursey 1973), we would maintain that more experimental work is needed to be sure of its efficiency in soft water, especially if the pH

is also low. In addition, the degree of gill hyperplasia at the end of the experiment was slightly higher in the chloramine-T-treated fish than in the malachite green–formalin-treated fish (in 2001) or in the Desirox–formalin-treated fish (in 2002). It was by no means at the risk level for the health of the fish, however (P. Koski et al. unpubl.).

A low safety margin between the effectiveness of potassium permanganate in controlling ichthyophthiriasis and mortality was found when channel catfish were treated daily over 10 d (Straus & Griffin 2002), and we also found that mortality was higher in the tanks treated with potassium permanganate than in those receiving chloramine-T or malachite green (0.5 vs. 0.1% at Farm B in 2001). No differences were found, however, between potassium permanganate, chloramine-T and malachite green–formalin, because the parasite burden was low at Farm B, nor were there any harmful effects on the fish gills (P. Koski et al. unpubl.).

Acknowledgements. We are most grateful to the staff of the fish farms for helping us to treat the fish as planned. We also thank the managing directors of the farms for providing us with laboratory facilities. The work was financed by the Ministry of Agriculture and Forestry in Finland.

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Editorial responsibility: Wolfgang Körting, Hannover, Germany

Submitted: June 3, 2004; Accepted: September 7, 2004
Proofs received from author(s): February 16, 2005