Ultraviolet irradiation inactivates the waterborne infective stages of *Myxobolus cerebralis*: a treatment for hatchery water supplies

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ABSTRACT: The effects of ultraviolet (UV) irradiation on the viability of the waterborne triactino-myxon stages of *Myxobolus cerebralis* were evaluated by vital staining and the infectivity for juvenile rainbow trout *Oncorhynchus mykiss*. A dose of 1300 mWs cm^{-2} was required to inactivate 100% of the triactinomyxons held under a static collimated beam of UV as determined by vital staining. Juvenile rainbow trout were protected from infections with *M. cerebralis* when exposed to 14 000 or 1400 triactinomyxon spores per fish that had been treated with the collimating beam apparatus (1300 mWs cm^{-2}). Among all fish receiving UV-treated triactinomyxons, none had clinical signs of whirling disease, or evidence of microscopic lesions or spores of *M. cerebralis* after 5 mo at water temperatures of 15°C. In contrast, 100% of the fish receiving the higher dose of untreated triactino-myxons developed clinical signs of whirling disease and both microscopic signs of infection and spores were detected in all of the high and low dose trout receiving untreated triactinomyxon expo-sures. Two additional trials evaluated the *Cryptosporidium* Inactivation Device (CID) for its ability to treat flow-through 15°C well water to which triactinomyxons were added over a 2 wk period. CID treatments of a cumulative dose exceeding 64 000 triactinomyxons per fish protected juvenile rain-bow from infections with *M. cerebralis*. Rainbow trout controls receiving the same number of untreated triactinomyxons developed both microscopic lesions and cranial spore concentrations up to $10^{4.6}$ per $\frac{1}{2}$ head, although no signs of clinical whirling disease were observed. UV (126 mWs cm^{-2}, collimated beam apparatus) was also effective in killing *Flavobacterium psychrophilum*, the agent causing salmonid bacterial coldwater disease, as demonstrated by the inability of bacterial cells to grow on artificial media following UV treatment.

KEY WORDS: Whirling disease · *Flavobacterium psychrophilum* · Ultraviolet · Disease control

INTRODUCTION

Whirling disease among salmonid fish is caused by the myxozoan parasite *Myxobolus cerebralis* (Hofer 1908). The disease, once thought to be problematic only among hatchery-reared salmonids (Halliday 1976, Hoffman 1990), has now been identified as a major cause of declines among certain wild trout populations in the intermountain west of the USA (Nehring & Walker 1996, Vincent 1996). The disease has been effectively managed in hatchery-reared salmonids by preventing or reducing the exposure of young susceptible fish to the infectious stages or tri-actinomyxons as released from infected oligochaetes (Hoffman 1990). This has been accomplished by rear-ing young fish in well water to avoid exposures to the parasite until fish are older and more resistant, or by modifying pond designs to minimize habitat for the oligochaete host (Schaperclaus 1986, Wolf 1986).

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While clinical disease manifestations may be controlled by these measures, fish are infected and the parasite develops to form spores in the skeletal elements. The stocking of subclinically infected fish is one principal method responsible for the spread of whirling disease (Hoffman 1970, Modin 1998).

The studies of Wolf & Markiw (1984) demonstrated the 2-host life cycle for the parasite and identified the previously unknown waterborne infectious stages of *Myxobolus cerebralis*: the triactinomyxons. The spore stages produced in the fish are extremely resistant to physical and chemical inactivation (El-Matbouli et al. 1992). In contrast, the triactinomyxon stages released into the water from infected oligochaetes are shorter lived and more susceptible to inactivation (Markiw 1992a). Removing or inactivating these waterborne infectious stages is viewed as an essential approach to controlling infections with *M. cerebralis* among salmonids when surface waters must be utilized for hatchery rearing. Hoffman (1974, 1975) demonstrated that ultraviolet light (UV) treatments were effective in protecting rainbow trout from whirling disease even though the waterborne infectious stages were at that time unknown. Hoffman (1974, 1975) found UV treatments at doses ranging from 18 to 112 mWs cm^{-2} provided partial to complete protection from *M. cerebralis* infections. These initial studies demonstrated the potential of UV treatments but did not establish minimum lethal doses required to inactivate the triactinomyxon form of *M. cerebralis.* In more recent years, sophisticated and much more powerful systems for treatment of water with UV (for municipal water supplies) have been developed (Campbell et al. 1995, Clancy et al. 1998, Bukhari et al. 1999). The *Cryptosporidium* Inactivation Device (CID) technology is one example of these newer approaches to the use of disinfection of water using UV light (Clancy et al. 1998). This device uses a combination of UV and nominal 2 µm sintered stainless steel screens for effective trapping and inactivation of the human pathogen *Cryptosporidium* in municipal water supplies. In this study we describe the use of a collimating beam apparatus to determine the dose response curves for the triactinomyxon stages of *M. cerebralis* as evaluated by vital staining and infectivity for rainbow trout *Oncorhynchus mykiss*. In addition, we examined the dose response of *Flavobacterium psychrophilum*, the agent causing salmonid bacterial coldwater disease, to UV irradiation (Bernardet et al. 1996). We then tested the efficacy of UV treatments as provided by the CID to inactivate triactinomyxons introduced into a flow-through system to aquaria containing rainbow trout. The results of these studies and the application of these approaches to eliminate *M. cerebralis* from hatchery water supplies are described here.

METHODS

Laboratory propagation of parasite stages. The triactinomyxons of *Myxobolus cerebralis* were propagated in the laboratory from known susceptible oligochaete populations infected with spores extracted from rainbow trout *Oncorhynchus mykiss* tissues by the plankton centrifuge procedure as described by Andree et al. (1998). Triactinomyxons from infected oligochaete populations were harvested every 2 to 3 d as required for evaluating the efficacy of UV light treatments in destroying the infectivity of the triactinomyxons for fish. Two UV inactivation procedures were employed, the first utilized a collimating beam apparatus for establishing dose response curves by vital staining and destruction of infectivity for rainbow trout (static UV treatments). The second procedure involved continuous treatments of large water volumes with the CID, a unit designed to treat municipal water supplies (Clancy et al. 1998). During flow-through trials with the CID, triactinomyxons were periodically added to the water supply before entering the CID; treated water was dispensed to tanks containing young rainbow trout.

UV dose response curves—collimating beam. A collimating beam apparatus (kindly supplied by Safe Water Solutions L.L.C., Scottsdale, Arizona) with 4 identical beams of UV light from 1 light tube was used to direct UV light onto 4 petri dishes mounted on insulated magnetic stir plates (Bukhari et al. 1999). The intensity of the UV irradiation on bisecting *x*- and *y*-axes of the dishes was measured with a radiometer and sensor (Bukhari et al. 1999). The measured values were used to calculate an average irradiance over the surface of the dish.

The effects of UV exposure on triactinomyxons suspended in 20 ml of water in each petri dish under 3 of the replicate UV beams was compared with 2 control dishes shielded from the radiation. The solutions were mixed gently throughout the treatment with magnetic stir bars. In the initial dose response experiments 0.1 ml aliquots were removed from each dish at selected time points for later analysis by vital staining. The appropriate dosage for complete inactivation of the triactinomyxons was then determined. Subsequently, larger numbers of parasites were irradiated and tested for their ability to infect young rainbow trout.

Vital staining of triactinomyxons. An adaptation of the vital stain originally used for triactinomyxons of *Myxobolus cerebralis* by Markiw (1992b) and as described by Wagner (1998) was used to determine potential viability of the parasite after exposure to UV irradiation. A 100 µl aliquot from the parasite suspension was mixed on a microscope slide with 50 µl of a

52 mg l^{-1} propidium iodide solution and 50 µl of fluorescein diacetate (100 µl of 5 mg ml^{-1} stock solution added to 8.3 ml water). A coverslip was added and the slide was held at least 45 min at 4°C before examination with a microscope equipped with the proper excitation and barrier filters. Under these conditions viable sporoplasm cells in the triactinomyxons stained green while those that were inactivated stained red.

Effects of static UV treatments on infectivity for trout. Triactinomyxons for the fish infectivity study were aliquoted to 8 petri dishes: 4 dishes with 1.4×10^6 and 4 with 1.40×10^5 triactinomyxons provided a low (1400 per fish) and high (14 000 per fish) dose exposure of rainbow trout. Two dishes from each group were treated with UV for the equivalent of 1300 mW s cm^{-2} at 20°C. The irradiance under the beam was 135 µW cm^{-2}; therefore, approximately 3 h was required to achieve the needed dose. Two dishes from each group were held under the same conditions but did not receive UV treatment (Low and High). Groups of 40 rainbow trout (0.2 g) were then exposed to the contents of each petri dish representing the 4 treatment groups in 15°C well water. An additional 2 groups of 40 fish received no exposures to triactinomxyons (unexposed control). The fish were then transferred to 20 l aquaria receiving 15°C well water at a rate of 3 l min^{-1}. Details on the exposure protocols and fish maintenance are described by Hedrick et al. (1999a). After 5 wk, 5 fish from each unexposed group and each group receiving high dose triactinomyxon exposures with or without UV treatments were removed and examined by PCR for the presence of *Myxobolus cerebralis* infection (Andree et al. 1998). At 5 mo post-exposure the experiment was terminated. Ten fish from each tank were examined individually for spore concentrations in cranial cartilage and microscopic lesions in stained tissue sections associated with *M. cerebralis* (Thoesen 1994) as described by Hedrick et al. (1999b). Remaining fish in all groups exposed to UV-treated parasites were analyzed as 5 fish pools for the presence or absence of the spores in cranial cartilage.

Effects of CID UV treatments on infectivity for trout. The CID unit was installed directly into the Fish Disease Laboratory at the University of California Davis such that all water delivered to six 130 l tanks received treatment prior to contacting the fish. The CID unit irradiates water that flows through its 2 µm filters, and particulates trapped on the filter are treated with high-energy UV irradiation. The filtration and irradiation cycles between 2 chambers, and filters are back flushed at the end of each cycle to maintain flow rates. During triactinomyxon trials, the flow of 15°C well water through the machine was 18 l min^{-1}, with 3 l min^{-1} flowing into each of the 6 test aquaria. At all other times the flow through the machine was 131 l min^{-1}, with flow in excess of the 18 l min^{-1} bypassing the fish tanks into a recirculating loop with a 650 l circular holding tank.

To introduce parasites into the treatment system, tractinomyxons were suspended in 4 l of water and metered into the intake pipe of the CID with a pump over a period of approximately 12 min. This time frame overlapped the cycle between the 2 chambers of the filtration-irradiation units to ensure that parasites were flowing through the complete range of the machine's function. The UV dosage on the CID was set at 4000 mW s cm^{-2} per chamber, a significant excess over the 1300 mW s cm^{-2} actually required for inactivation of the triactinomyxons. The design of the CID ensures that organisms trapped temporarily on the screens before passing through the machine receive a minimum of 4000 mW s cm^{-2} and a maximum of 8000 mW s cm^{-2}.

For testing of the CID with parasite challenge studies, 13 rainbow trout (2.4 g) were placed in 2 l of water in each of the 6 tanks supplied with water treated by the CID. Water supply to the tanks was arrested for 5 min after the addition of the parasites to the CID to provide time for passage of the parasites through the machine; then water flow to each tank was resumed at a rate of 3 l min^{-1}. Parasites were introduced into the CID over a 12 min period. A period of 19 min was required to bring the volume to capacity in the 130 l tanks. Water flow into the tanks was stopped when full, and tanks were held with aeration but without water flow for 14 h to maximize contact between parasites in the aquaria. Flows were resumed the next day, and fish were maintained as previously described. Untreated triactinomyxon control exposures were designed to mimic conditions of the CID treated exposures. Thirteen fish were placed in 2 l of water in similar tanks, with water inflow set at 3 l min^{-1}. An amount of parasites equal to $\frac{1}{6}$ of that injected into the CID unit was added to each tank. Water flow was stopped when tanks were full. Tanks were held static overnight, with flows returned to 3 l min^{-1} to each tank the next day. A total of 64 200 triactinomyxons per fish was added through a series of 4 exposures over a 2 wk period to fish in both the CID and untreated water groups. A group of fish held under the same conditions but receiving only well water and no parasites served as unexposed controls. The well water temperature throughout the trial was maintained at 15°C. The study was conducted in the winter such that water temperatures would not increase or decrease significantly during static water periods of exposure. The fish were maintained as described previously (Andree et al. 1998). Five fish from replicate unexposed, CID exposed, and exposed untreated aquaria were examined by PCR analysis at 5 wk post-exposure (Andree et al.

1998). In addition, 5 fish from replicate unexposed, exposed untreated aquaria and 10 fish from each CID exposed aquaria were examined for cranial spore concentrations and microscopic lesions at 5 mo post-exposure. Two separate trials (Trials 1 and 2) were conducted following this procedure with the only difference that fish in Trial 1 received a cumulative dose over a period of 2 wk of 7539 triactinomyxons compared to 64 200 triactinomyxons per fish in Trial 2.

UV dose response curves for *Flavobacterium psychrophilum.* An additional study conducted with the UV collimating beam apparatus examined the inactivation of the bacterial pathogen *Flavobacterium psychrophilum*. The bacterium was grown in broth (Anacker & Ordal 1955) at 15°C for 72 h. Broth cultures were then distributed into 3 aliquots of 20 ml in sterile petri dishes. Two replicates were exposed to UV irradiation (140 μW cm^{-2}) and 1 dish was not irradiated (control). Aliqouts of 0.5 ml were removed at selected exposure times from all 3 plates, suspended in Anacker and Ordal broth for serial dilutions and spread onto tryptone yeast extract (TYES) agar for enumeration of viable cells. Colonies were counted after 7 d incubation at 15°C.

RESULTS

UV dose response curves — vital staining

A cumulative dose of 1300 mWs cm^{-2} was required to inactivate the triactinomyxons as suspended in well water under the conditions tested (Fig. 1). The sporo-
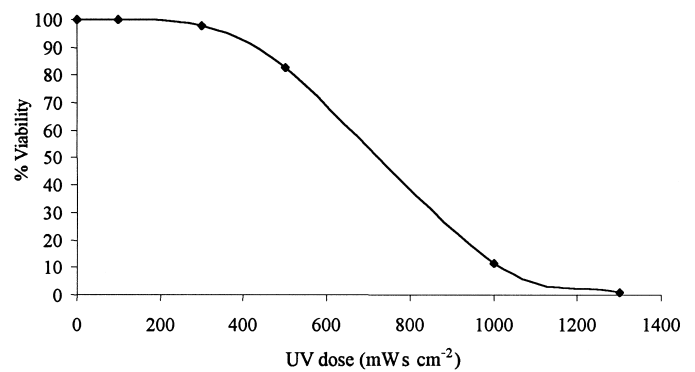

Fig. 1. Effects of cumulative dose of ultraviolet light on the viability of triactinomyxon stages of *Myxobolus cerebralis* as determined by vital staining

plasm cells in the apical end of the triactinomyxon all stained red after treatment at this dosage, suggesting that these cells were no longer viable. In contrast, 57 % of the untreated triactinomyxons held under the same conditions contained sporoplasm cells all of which stained green, suggesting they were viable. Using the cumulative dose of 1300 mWs cm^{-2} as an estimate, trials with larger numbers of triactinomyxons were conducted for the subsequent fish exposure studies.

Effect of static (UV collimating beam) treatments on infectivity for trout

At 5 wk post-exposure and prior to the onset of clinical signs, all 10 fish receiving the high dose of untreated triactinomyxons were positive, while those fish receiving the UV-treated triactinomyxons were all negative (PCR test). In addition, 10 unexposed, untreated control fish were also negative. Clinical signs including black tail and whirling swimming first became evident at 6 wk following exposure to triactinomyxons in the high dose not treated with UV; by 2 mo 100 % of the fish in the high dose group and 13 % in the low dose group displayed clinical signs (Table 1). No clinical signs were observed among fish in any groups receiving UV-treated triactinomyxons. At 5 mo post-exposure, none of the trout receiving either the high or low dose triactinomyxons treated with UV had microscopic lesions or spores (Table 1). In contrast, 100 % of the fish exposed to triactinomyxons not treated with UV

Table 1. Effects of static ultraviolet light treatments with the collimating beam apparatus on the infectivity of 2 concentrations of triactinomyxons (High and Low) for juvenile rainbow trout. nd: no spores detected

| | Triactinomyxon exposure | | | | |
| | UV-treated | | No treatment | | |
Parasite exposure dose[a]:	High	Low	High	Low	None
Presence of clinical signs[b]	0/64	0/69	54/54	8/62	0/66
Prevalence of infection[c]	0/20	0/20	20/20	20/20	0/20
Mean lesion score[d]	0	0	3.0	2.2	0
Mean spore count[e]	nd	nd	$10^{5.6}$	$10^{4.6}$	0

[a]Dose of triactinomyxon stages of *Myxobolus cerebralis* per fish used in the exposures for High was 14 000 and Low 1400 triactinomyxons per fish

[b]Presence of black tail or whirling swimming at 2 mo post-exposure

[c]Infection with *M. cerebralis* was determined by presence of spores in the pepsin trypsin digestion of $\frac{1}{2}$ of the head of 20 individual fish (10 from each of 2 replicate groups per treatment) at 5 mo post-exposure

[d]Microscopic lesions were scored on a scale of none (0) to severe (5) among 10 fish in the high exposure group at 5 mo post-exposure

[e]Mean concentration of spores (per $\frac{1}{2}$ head) among infected fish in each group at 5 mo post-exposure

had microscopic lesions in the cranium and gills, with mean overall severity scores of 3.0 (high triactinomyxon exposure) and 2.2 (low triactinomyxon exposure). Mean spore concentrations for fish in the high and low group exposure to untreated triactinomyxons were $10^{5.6}$ and $10^{4.6}$ per $\frac{1}{2}$ head, respectively (Table 1).

Efficacy of CID UV treatments on infectivity for trout

Two trials conducted with the CID provided similar results demonstrating the efficacy of this water treatment in eliminating infectivity of the triactinomyxon stage of *Myxobolus cerebralis* from water supplied to juvenile trout. At 5 wk post-exposure, prevalence of PCR-positive fish in groups receiving untreated triactinomyxons was less in Trial 1 (1/6) than in Trial 2 (6/6). In both trials fish receiving CID-treated triactinomyxons were PCR-negative (0/6). In addition, 6 unexposed, untreated control fish in both Trials 1 and 2 were also PCR-negative. But for 1 fish in the untreated triactinomyxon-exposed groups in Trial 2, no clinical signs were observed during the course of either trial. In both trials at 5 mo post-exposure, none of the 20 fish sampled from the CID-treated groups had microscopic lesions or spores (Table 2). In contrast, 100% of the fish receiving untreated triactinomyxons contained microscopic lesions in the cranium and gills, with a mean score of 2.4 in Trial 2. No microscopic pathological examinations were conducted in Trial 1. The mean spore concentrations per $\frac{1}{2}$ head for the untreated triactinomyxon group was greater in Trial 2 than in Trial 1.

Table 2. Efficacy of the *Cryptosporidium* Inactivation Device (CID) in protecting juvenile rainbow from exposures to triactinomyxons of *Myxobolus cerebralis*. nd: no spores detected

| | Triactinomyxon-exposed[a] | | | | Unexposed controls | |
| | CID-treated | | No treatment | | | |
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Prevalence of infection[b]	0/20	0/20	18/20	20/20	0/20	0/20
Mean lesion score[c]	–	0	–	2.4	0/20	0/20
Mean spore count[d]	nd	nd	$10^{4.4}$	$10^{4.6}$	nd	nd

[a]Fish were exposed to a cumulative dose of 7539 (Trial 1) or 64 200 (Trial 2) triactinomyxons per fish over a 2 wk period

[b]Infection with *M. cerebralis* was determined by presence of spores in the pepsin trypsin digestion of $\frac{1}{2}$ of the head of 20 individual fish (10 from each of 2 replicate groups per treatment) at 5 mo post-exposure

[c]Microscopic lesions were scored on a scale of none (0) to severe (5) among 10 fish in the high exposure group at 5 mo for Trial 2 only

[d]Mean concentration of spores among infected fish in each group at 5 mo post-exposure

Table 3. Effects of static ultraviolet light treatments with the collimating beam apparatus on the viability of *Flavobacterium psychrophilum*. The numbers of viable bacteria are expressed as colony forming units ml^{-1}

| Time (min) | Untreated control | Treated with UV irradiation | |
		Replicate 1	Replicate 2
0	2.2×10^5	1.0×10^5	6.0×10^4
5	2.6×10^5	1.0×10^5	5.0×10^4
15	2.3×10^5	0	0
30	2.2×10^5	0	0

UV dose response curves for *Flavobacterium psychrophilum*

Treatments of suspensions of *Flavobacterium psychrophilum* with UV irradiation were ineffective at doses of 42 mW s cm^{-2} (Table 3). However, at doses of 126 or 252 mW s cm^{-2} bacterial cells were killed by the treatment (Table 3).

DISCUSSION

UV irradiation was effective in destroying the viability of the triactinomyxon stages of *Myxobolus cerebralis* as determined by both vital staining and elimination of infectivity for young rainbow trout. UV treatments were highly effective whether administered in small volumes with a low pressure collimating beam apparatus, or in larger volumes with higher intensity radiation in a flow-through device (CID) designed for water supplies. These results demonstrated the feasibility of UV irradiation, even at larger scales needed for hatchery water supplies, to prevent infections of hatchery reared trout with *M. cerebralis*. In addition, the UV dose effective against *M. cerebralis* also inactivated the bacterium *Flavobacterium psychrophilum*, the cause of salmonid bacterial coldwater disease.

UV irradiation has been most commonly used to eliminate or reduce pathogens in closed recirculating fish rearing units but also in larger scale fish production units using single-pass water (Hoffman 1974). The principal mode of action for UV irradiation, particularly that in the 220 to 300 nm wavelengths, is the formation of pyrimidine dimers in the target cell DNA

(Schechmeister 1991). Among the many stages of *Myxobolus cerebralis*, the most susceptible form to both chemical and physical destruction is the triactinomyxon, the form released into the water from the oligochaete host (Markiw 1992b). This is the form present in the water that effectively attaches to the epidermis of the fish and then proceeds to invade through the skin, migrating to the nerves, and finally to the cartilage (Markiw 1989, El-Matbouli et al. 1995). This stage of the parasite is large (up to 180 µm between valve tips) and possesses relatively thin valves as protection for the approximately 64 sporoplasm cells that are packaged for delivery into the fish epidermis (El-Matbouli et al. 1999). Although unknown at the time, the first trials with UV with *M. cerebralis* by Hoffman (1974, 1975) were targeting these triactinomyxon stages. Hoffman (1974, 1975) recommended treatments of 35.0 mWs cm^{-2} for the elimination of *M. cerebralis* infectivity from water supplies. He found that treatments of 18.0 to 27.7 mWs cm^{-2} gave less consistent results, protecting fish from clinical disease but not all fish from infections.

In our trials with the collimated beam apparatus, levels of 1300 mWs cm^{-2} were required to inactivate triactinomyxons as judged by the lack of viable staining of all of the sporoplasm cells in all of the triactinomyxons. This dosage is probably in excess of that required as it may not be necessary to inactivate all the sporoplasm cells to render the triactinomyxon noninfectious. This in part may explain why much lower doses were effective in the trials by Hofmann (1974, 1975).

Treatments of larger volumes of water in hatchery water supplies, particularly during early fish rearing, is viewed as one possible means to aid in the control of whirling disease. While the flow rates examined in our study were below the capacity of the apparatus, the CID effectively eliminated infectivity of triactinomyxons as administered in repeated dosages over a 2 wk period in 2 separate trials. In the CID, water passing through the 2 µm screen receives a dose of 4000 mWs cm^{-2} and materials temporarily trapped on the filter can be treated at even higher doses up to 8000 mWs cm^{-2}. These doses of UV irradiation are well above the 1300 mWs cm^{-2} determined from the static trials to eliminate all infectivity due to *Myxobolus cerebralis*. We presume, but did not test, that the physical forces during the temporary filtration experienced by triactinomyxons entering the CID could contribute to shearing of the parasite that might also greatly reduce infectivity. We have found that although triactinomyxons are large when fully extended (up to 180 µm) they can fold upon themselves and effectively pass through screen sizes of 20 µm (authors' unpubl. data). However, triactinomyxons entering a 2 µm screen under pressure

would be retained and only sporoplasm stages, which presumably are noninfectious once released from the surrounding membrane, would pass the screen (El-Matbouli et al. 1999). Larger models of the CIDs are available with capabilities to treat up to 3344 l min^{-1}, and these could be used for early rearing of salmonids to prevent serious infections with *M. cerebralis*. At one local hatchery, we estimated that 2 larger CID would be sufficient to rear 200 000 steelhead trout through the first 4 mo after hatching.

UV irradiation was also effective in destroying the salmonid bacterial pathogen *Flavobacterium psychrophilum*, a pathogen often occurring in the same hatcheries as whirling disease. Furthermore, this bacterium is prevalent among salmonid fish throughout the world and a major cause of losses among hatchery-reared rainbow trout (Bernardet et al. 1996). The bacterium is transmitted through the water column from fish to fish and presumably with eggs from infected adult fish (Brown et al. 1997). The bacterium as shed into the water would pass the 2 µm screen in the CID but the 4000 mWs cm^{-2} dose experienced as it passed the filter is well in excess of the 126 mWs cm^{-2} dose shown to inactivate over 10^5 colony forming units of the bacterium in our trials (Table 3).

Certain mechanisms for the transmission of whirling disease remain unknown, but stocking or transfer of live infected fish to new locations is clearly one known mode (Modin 1998). When the suitable oligochaete and fish hosts and environment are present, whirling disease can thrive. If these conditions are not met, the disease may fail to be established or remain at a low level that in part may explain the differential impacts of whirling disease in the intermountain west compared to other eastern and western states (Hedrick et al. 1998). Groundwater should be used whenever possible for hatchery-rearing of salmonids in whirling disease enzootic waters (Hoffman 1990), but when this is not possible, treatment of the incoming surface water with UV is a feasible alternative to prevent infection with *Myxobolus cerebralis*. Our controlled laboratory trials and those of Hoffman (1974, 1975) demonstrate that UV irradiation, particularly when delivered by newly designed and more efficient apparatuses, should be seriously considered for hatchery programs aimed at stocking fish when surface waters contain the infectious stages of *M. cerebralis*.

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