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Ultrasonic water treatment as an alternative means of controlling fish mortality caused by *Bucephalus polymorphus* cercariae

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Abstract

In laboratory and field trials conducted in the course of this study, ultrasonic treatment of water was shown to sigificantly reduce survival of cercariae of the species *Bucephalus polymorphus* Baer, 1827. Under laboratory conditions, exposure of a total of 480 cercariae to ultrasonic waves with a specific energy intake ranging from 0.000508 to 0.00176 kWh/L resulted in damage or death of the majority of exposed parasites. In field experiments carried out over a period of 20 days with common carp (*Cyprinus carpio*), ultrasonic treatment of water (0.000652 kWh/L) led to significant reductions in both prevalence and mean density of infection. In the control group provided with untreated water, prevalence and mean density were 76.3% and 0.26 metacercariae/g filet and host, and 0.12 metacercariae/g gill-tissue and host, respectively, whereas a prevalence of 26.4% and mean densities of 0.02 metacercariae/g filet and host, and 0.04 metacercariae/g gill-tissue and host were noted in the test fish which were exposed to ultrasonic treated water. However, energy costs rise in proportion to the amount of water treated, which might render the method discussed cost-prohibitive for large facilities, despite technical advances.

Introduction

In the European Union, legal administration of antiinfective drugs in commercial fish production has been gradually restricted in the recent past (Anonymous, 1990). In Germany, since January 2000 a medication licensed to treat endoparasites of commercial fish is unavailable, rendering afflicted facilities virtually helpless in their attempt to continue production in an economic mode. If these infections remain unchecked, parasites involved are likely to multiply exponentially, resulting in ever increasing fish mortality rates and, ultimately, in the production site becoming uneconomic. Alternative nonchemical methods to control and eliminate pathogenic organisms thus need to be found to ensure a continued cost-effective rearing of fish for human consumption.

In 2002, a fish-rearing and stocking company in southeastern Germany reported to be suffering reoccurring losses of fish during the summer months, with mortality rates of certain species on occasion reaching up to 100% (e.g. rainbow trout, *Oncorhynchus mykiss*, and common carp, *Cyprinus carpio*). The facility draws its water from a nearby reservoir, which, in accordance with its hypertrophic state, harbors vast amounts of molluscs, cyprinids and predatory fish, which potentially act as intermediate and final hosts for many metazoan parasites. The proprietor of the company suspected that the involved pathogens may be invading the fish farm with the reservoirs' water. On examination of various fish, the trematode *Bucephalus polymorphus* was quickly found to be causing the aforementioned losses, with metacercariae being embedded by thousands in the skin, musculature and especially the gills. Fish mortalities and pathogenic effects such as skin lesions, hemorrhage, necrosis and gill damage caused by this parasite have repeatedly been described in the scientific literature (Baturo 1978, 1980; Grabda and Grabda, 1967; Hoffmann et al., 1990). The life-cycle of *B. polymorphus* comprises three hosts: first, the zebra mussel (*Dreissena polymorpha*), where production of infective free-living cercariae takes place; and second coarse fish (mainly cyprinids) where metacercariae develop within cysts. Final hosts are predatory fish (such as pike-perch, *Sander luciopercae*, European eel, *Anguilla anguilla* and European catfish, *Silurus glanis*), which become infected by feeding on infected coarse fish (Baturo 1978). Miracidia, which hatch from eggs produced by the adult trematode living in the final hosts' digestive tract, will in turn infect zebra mussels.

In the search for an effective method to control larval bucephalosis, effects of ultrasonic waves on *B. polymorphus* cercariae (Figure 1) were evaluated. Specifically, laboratory experiments were conducted to test whether ultrasound affects survival of *B. polymorphus* cercariae and to determine the specific energy input necessary to cause pathogenic effects to the free-living parasite larvae. Moreover, field experiments were carried out to examine the practicability of this method under natural conditions. The results of these investigations are presented in this paper.

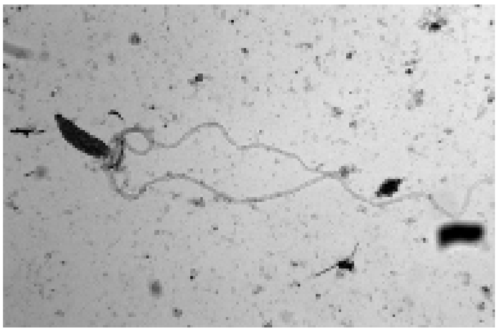

Figure 1. *Bucephalus polymorphus* cercaria with its highly contractile, bifurcated tail (mean body length (± S.D.) 201.3 (42.0) µm, n = 11).

Materials and Methods

Laboratory tests

To document the impact of ultrasonic energy on larval stages of *B. polymorphus*, groups of cercariae were exposed to continuous ultrasonic waves of different amplitude, using the ultrasound unit UP200S (Dr. Hielscher GmbH, Germany) in conjunction with two different sonotrodes (S14, S40) with 1.4 cm^2 and 4.0 cm^2 sonotrode-surface, respectively.

For the individual experiments, 10 viable cercariae randomly taken from sporocysts of three naturally infected *D. polymorpha*, were transferred into a glass containing 50 ml of tapwater. The glass was placed under the sonotrode, which was then lowered 1 cm into the liquid leaving a 1 cm space between the sonotrode and the bottom of the glass. The

Test No.	Sonotrode type	Amplitude (%)	Exposure time (s)	Mean energy input (W)	Specific energy input $W_{spec.}$ (kWh/L) [1]
1	S14	100	3	59.6	0.000985
2	S14	60	3	38.9	0.000649
3	S40	100	3	105.7	0.00176
4	S40	60	3	64.9	0.00108
5	S40	35	3	38.8	0.00064
6	S40	20	3	33.6	0.00056
7	S40	100	2	96.8	0.00107
8	S40	100	1	91.5	0.000508

Table 1. Parameters of laboratory exposure of *Bucephalus polymorphus* cercariae to ultrasonic waves.

liquid containing the cercariae was then treated for different time periods with various ultrasonic amplitudes (Table 1). After exposure, the liquid was carefully poured into a petri dish and cercariae were examined for possible damage using a microscope. Depending upon their condition, the cercariae were assigned to one of four defined groups (unharmed, damaged, killed but mainly intact, killed and fully destroyed). Each test was repeated six times generating a total of 60 cercariae per experiment. All tests were performed at room temperature.

Field tests

Based upon findings derived from laboratory tests, the practicability of ultrasonic water treatment for prevention of cercarial invasion was tested under field conditions at the afflicted fish farm. In August 2003, two fish tanks containing 1040 litres of water each were stocked with 38 (control group) and 34 (test group) common carp (*C. carpio*, mean body weight and length (± S.D.) were 260.7 (63.5) g and 25.6 (2.0) cm, respectively), which were all free of *B. polymorphus* infections prior to the experiments. Over a period of 20 days, both tanks received 60 L/minute of fresh water coming from the infected reservoir. During this time water parameters were as follows:

Temperature (°C):	17.1 - 24.1
Oxygen (mg/L):	4.4 - 7.6
pH:	8.8 - 9.0
NH_4 (mg/L):	0.3 - 0.4
NO_2 (mg/L):	0.01 - 0.05

Using the ultrasound unit UIP4000 (Dr. Hielscher GmbH, Germany), all water supplying the test groups' tank was treated continuously with ultrasonic waves generated by a generator running at 2350 W. The resulting specific energy input was 0.000652 kWh/L of water. Fish in the control group were provided with untreated water only. During the test period, all fish were fed with commercial fish pellets. Losses of fish were documented on a daily basis.

After 20 days, all fish were removed from the tanks and were transferred to holding facilities at the Institute for Inland Fisheries where they were held separately for a further 14 days to ensure complete development of the parasites to the metacercarial stage (Baturo, 1977). After that period, the fish were caught, stunned by a sharp blow to the head and killed (Amlacher, 1992; Anonymous 1993) for parasitological examination. Further dissection for metacercarial infections followed the recommendations given by Schuster et al. (1998). Briefly, 10-20 grams of filet with attached skin and fins were cut into cubes of approximately 1 cm^3 and then transferred into a glass holding 50 ml of a digestive solution consisting of 0.7 Vol.% hydrochloric acid (32%) and 0.9 Vol.% pepsin (2000 FIP U/g). Similarly, the gills were removed from the fish and, after weighing them; they were transferred into a separate glass with the digestive solution. The samples were then stirred at $120 min^{-1}$ for 90 min at room temperature using the magnetic stirrer Mini-MR™ (Jahnke & Kunkel, Staufen, Germany). Following digestion, the samples were sieved to remove scales and large debris. The sample was allowed to stand for ten minutes, causing the metacercariae to settle to the bottom of the glass, then all but the bottom layer of the liquid (approximately 10 ml) was poured off. The remaining liquid was transferred into a petri dish and then carefully swirled with a circular motion to concentrate all metacercariae in the centre of the dish. Under a stereo microscope, liquid was carefully removed from the edge of the dish until all metacercariae could easily be identified and counted. Depending on the cloudiness of the liquid, the last step of the process was repeated after adding some tapwater to the dish. Determination of parasites was based on the key of Bauer (1987).

Prevalence (i.e. the percentage of infected fish from the total number of fish examined) and mean density (i.e. the mean number of parasites per gram filet and gill tissue, respectively, per host examined) were used for a quantitative description of infection (Bush et al., 1997). Differences in prevalence and mean density of infection between the groups were tested by means of the Chi-Square test (Pearson) and the Mann-Whitney-U test, respectively. Results were considered significanct at $p<0.05$.

Results

Laboratory tests

Microscopic examination of exposed cercariae revealed strong damaging effects of ultrasonic waves using either of the two sonotrodes. At low specific energy inputs (tests no. 2, 5 and 6), the parasites would usually lose one or both of their furcae while remaining vital for a prolonged period of time, in some cases up to two hours. An increase of ultrasound amplitude and/or time of exposure led to punctuation of the parasites' integument and

Figure 2. Dead but mainly intact *Bucephalus polymorphus* cercaria after exposure to ultrasonic waves with a specific energy input of 0.000508 kWh/L (test no. 8).

Test no.	Number of killed and fully destroyed cercariae (Mean ± S.D.)	Number of killed but mainly intact cercariae (Mean ± S.D.)	Number of damaged cercariae (Mean ± S.D.)	Number of unharmed cercariae (Mean ± S.D.)
1	0	8.6 (1.0)	1.3 (1.0)	0
2	1.0 (1.0)	6.5 (0.5)	2.0 (0.8)	0.5 (0.8)
3	5.3 (2.5)	4.7 (2.6)	0	0
4	5.0 (1.8)	4.5 (2.4)	0	0.5 (0.8)
5	3.5 (3.2)	3.7 (2.9)	0.6 (0.8)	2.1 (1.8)
6	1.5 (1.6)	4.5 (1.0)	0.3 (0.8)	3.6 (1.0)
7	3.1 (1.7)	5.7 (2.6)	0.3 (0.8)	0.8 (0.75)
8	1.5 (1.3)	7.2 (1.1)	0.5 (0.5)	0.8 (0.7)

Table 2. Number of killed and fully destroyed, killed but mainly intact, damaged, and unharmed *Bucephalus polymorphus* cercariae after ultrasonic treatment with the UP200S ultrasound unit.

caused tissue and body liquids to escape through the skin ruptures (compare Figures 1 and 2). Affected parasites died within minutes. Further increase in specific energy input (test no. 3) resulted in almost instant destruction of the cercariae. Following exposure, scattered organic debris filled the microscopic field of view.

The number of unaffected cercariae was reduced with both increased amplitudes and increased exposure times (Table 2).

A significant positive correlation (r = 0.767; p = 0.026) was found between the specific energy input and the number of "killed and fully destroyed" and "killed but mainly intact" cercariae (Figure 3).

Field tests

During performance of the field experiments no mortalites were observed in either of the two groups. On examination, 76.3% of carp in the untreated control group were diagnosed with bucephalosis. Mean densities were 0.26 metacercariae/g filet and host, and 0.12 metacercariae/g gill tissue and host, respectively. Of the carp receiving ultrasonic-treated water, only 26.4% carried encysted metacercariae, mean densities were 0.02 metacercariae/g filet and host, and 0.04 metacercariae/g gill tissue and host. The differences between the control and the test group were statistically significant for both prevalence (p < 0.001) and mean densities (p = 0.006 (gills) and p < 0.001 (filet)).

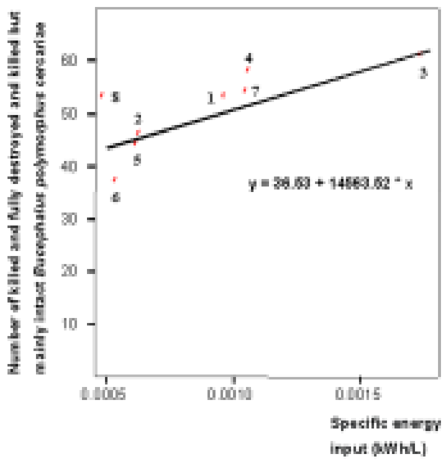

Figure 3. Association between specific energy input and number of killed and fully destroyed and killed but mainly intact *Bucephalus polymorphus* cercariae.

Discussion

The application of ultrasonic energy as a means to fight *B. polymorphus* cercariae was shown to be effective in both laboratory settings and under field conditions. The results show that ultrasonic waves either directly destroy or damage cercariae, leading to significant reductions of cercarial infections in fish. The destructive effect of ultrasonic waves in water is based on small cavitational bubbles, which are formed by fast longitudinal movements of the sonotrode. The bubbles implode forcefully, locally causing high differences in pressure as well as high temperatures, thus disrupting cells and tissue in their immediate vicinity (www.hielscher.com). Laboratory experiments revealed that, depending upon specific energy input, cercarial integrity was significantly impaired by ultrasonic treatment. Low energy inputs usually resulted in separation of one or both of the furcae whereas application of high energy inputs caused complete cercarial destruction as shown by bodily remnants found in the liquid. The specific energy input in this matter determines the efficacy of ultrasonic treatment rather than the type of sonotrode used (see tests 2 and 5). An increase in specific energy input could be achieved by either enhancement of the amplitude (and thus energy input) or, to a certain extent, by prolongation of exposure time. All tests performed reduced the number of surviving individuals, while test no. 3, followed by tests no. 4 and 7, caused highest mortality rates. However, in these cases high mortalities were achieved by utilizing large amounts of energy. When estimating the effectiveness of ultrasonic manipulation by comparison of the specific energy input with the percentage of "killed but mainly intact" and "killed and fully destroyed" parasites, test no. 8, followed by no. 2 and 5, revealed the best results (i.e. produced a reasonable number of affected cercariae with a moderate energy input).

The field experiments indicated that, in general, ultrasonic treatment of water can be used as a means to impair infectivity and / or survival of *B. polymorphus* cercariae resulting in reduced prevalence and density of infection in fish. In carp which received treated water, prevalence was almost three times lower than in fish in the untreated control group, while mean densities were reduced by up to 93 %.

Bucephalus polymorphus is widespread among fish populations of European waters (Baturo, 1977; Pojmanska, 1985; Taskinen et al., 1991; Wierzbicka et al., 1982; Odening, 1990; Schuster et al., 1998; Diler and Yildirim, 2003) and has been reported as causative agent of diseases and mortality (Baturo, 1978, 1980; Grabda and Grabda, 1967; Hoffmann et al., 1990; Paperna, 1996). The distribution of this trematode is facilitated by the invasive nature of its first intermediate host, the zebra mussel *D. polymorpha*, which is quickly conquering new habitats worldwide. Moreover, since this parasite has a low host specifity for both its second intermediate and definitive hosts, further expansion is promoted. Therefore, additional new host records must be expected on a global scale (Diler and Yildirim, 2003).

Under laboratory conditions, successful ultrasonic treatment of various free-living parasite stages was carried out by Kamenskij (1970), who was able to kill eggs, miracidia and cercariae of the diplostomatid trematodes *Diplostomum spathaceum* and *Tylodelphys* *clavata*. Regretfully, the precise mean energy inputs employed in those experiments are not given, but estimates from the provided data suggest them to be in the range of approximately 120 to 300 W and thus to be significantly higher than in the experiments performed in the present study. Although far-reaching technical progress has been made in the development of sonotrodes during the last 30 years, the application of ultrasonic energy to fight cercariae infective to fish still remains expensive. Assuming electrical energy costs of 0.16 •/kWh and using the specific energy input of 0.000652 kWh/L employed in the field trials, treatment of 1m^3 of water/min results in costs of 6.25 •/h, which totals 4,500 •/ month. Even when the low specific energy input of 0.000508 kWh/L, applied in the most efficient laboratory test (no. 8), is used to calculate costs for treatment of 1m^3 of water/ min, the monthly electrical bill would still amount to 3,511 •. Without taking into account the costs for acquisition and installation of an ultrasound unit, or costs for a regular substitution of worn sonotrodes, the results presented herein clearly demonstrate that antiparasitic treatment of large amounts of water using ultrasonic energy with an efficiency of nearly 100% will be restricted by financial considerations. However, in cases where a lower efficacy of treatment is sufficient, or where small aquacultural production subunits can be treated over restricted periods of cercarial invasions only, or where electrical current may be obtained from inexpensive sources, application of ultrasonic energy might be considered to reduce cercarioses to subclinical levels.

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