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The use of permitted ectoparasite disinfection methods on young pike-perch *(Sander lucioperca)* after transition from over-wintering lake to RAS

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Abstract. With the growing importance of recirculating aquaculture systems there is an increasing demand for proper parasite disinfection methods. It is especially true in cases, when the stocked fish comes from a natural environment to the RAS for rearing or experimental purposes. Our aim was to test some parasite disinfection methods on pike-perch (*Sander lucioperca*) stock (14.25 ± 0.45 cm, 20.57 ± 2.03 g), from a farm, where they were kept in over-wintering ponds. The experiment was conducted in a recirculating system with nine aquarium tanks (300 L each) and an overall volume of 4500 L, and in three separate round tanks (230 L). We used two UV lamps (18 watt each) in series to avoid re-infection between fish tanks. Five fish per group were sampled on the 0.-7th-14th days. Four treatments were replicated three times, as follows: 1) Control group, where no treatment was used (the three control groups were the three separate round tanks); 2) Continuous 0.5% salt (NaCl) treatment; 3) Combined bath treatment: 0.5% constant salt concentration along with 2 x 10 minutes, 2% salt short bath. This was applied directly after the first and the second sampling; 4) "Detox SA" treatment (active ingredients: 4.5% peracetic acid, 10% acetic acid, and 20% hydrogen peroxide), plus 0.5% constant salt concentration. The Detox treatment was used on the first day, and then daily with the concentration of 20 mL/m^3 (as recommended by manufacturer "ORPC"). The system was shut down for 40 minutes during the treatment. During the experiment, three groups of ectoparasites were found: *Trichodina sp*. *Gyrodactylus sp*. *Ichthyophthirius multifiliis*. The *Trichodina* infection cleared in all three groups, whereas in the control group the infection remained, however it declined. The *Gyrodactilus sp.* population with the Detox SA's treatment was completely eliminated, while the infection remained in the short bath treated group, again drastically reduced. In the groups of continuous 0.5% salt treatment the stock of *Gyrodactilus sp.* increased nearly 30 fold, therefore this treatment for this parasite was ineffective. In the control group over the first weekend of the herds it increased and then decreased. Serious *Ichthyophthirius multifiliis* infection developed only in the control groups, but not in any other group. This demonstrated that use of 0.5% continuous salt treatment alone could inhibit the propagation of *Ichthyophthirius multifiliis*. As a result of the experiment, the conclusion can be drawn, that a series of treatments for 10 or 14 days with the three combined methods can be successfully used for disinfection of fish originating from ponds.
Key Words: Trichodinosis, gyrodactylosis, ichthyophthiriosis, disinfection methods, pike-perch.

Kivonat. Az intenzív recirkulációs rendszerek előtérbe kerülésével egyre fontosabbá válik a megfelelő parazita-mentesítési eljárások kidolgozása. Ez különösen igaz azokban az esetekben, amikor természetes vízből mesterséges környezetbe szoktatjuk be a halakat továbbnevelési, vagy kísérleti céllal. Vizsgálatunk célja az volt, hogy egynyaras, telelőn tartott, legyengült süllő (*Sander lucioperca*) állományon (14.25 ± 0.45 cm; 20,57 ± 2,03 g) teszteljünk néhány parazita-mentesítő módszert. A vizsgálatot egy 9 kádas 4500 liter össztérfogatú recirkulációs rendszerben, illetve 3 külön álló, levegőztetett körkádban végeztük. A rendszerben 0,5%-os NaCl koncentrációt tartottunk fenn. A kísérlet 14 napig tartott. Kádanként 5-5 halat vizsgáltunk a kísérlet 0.-7.-14. napján. 4 kezelést alkalmaztunk 3 ismétlésben: 1) Kontroll csoport, amiben semmilyen kezelést nem használtunk. 2) 0,5%-os folyamatos sós (NaCl) kezelés. 3) Kombinált fürdetés: 0,5%-os folyamatos só-koncentráció + 2 x 10 perces 2 %-os sós fürdetés. 4) DETOX-SA 20ml/m^3/nap-os kezelés (gyártó ajánlásával), 0,5%-os folyamatos só-koncentráció mellett az első minta vétel után, amit 40 percig leállított rendszeren végeztünk. A kísérlet során 3 ektoparazita csoporttal találkoztunk: *Trichodina sp.*, *Gyrodactylus sp.*, *Ichthyophthirius multifiliis*. A *Trichodina sp.* fertőzöttség mindhárom kezelés kádjaiban megszűnt, míg a kontroll kádaknál mértéke visszaesett, de fennmaradt. A *Gyrodactilus sp.* állományát a DETOX-SA-s kezelés teljesen eltüntette, míg fürdetéses kádakban a fertőzöttség megmaradt, de drasztikusan visszaesett. A hosszú sós kezelés kádjaiban a hámféreg állomány közel 30 szorosára nőtt. A kontroll kádakban az első hét végére számuk

emelkedett, majd visszaesett. Komoly *Ichthyophthirius multifiliis* fertőzés egyedül a kontroll kádaknál alakult ki. Ez azt jelenti, hogy felszaporodását már a 0,5%-os folyamatos sós kezelés önmagában is meggátolta. A kísérlet eredményeként azt az ajánlást tehetjük, hogy egy 10-14 napos kezelés sorozat segítségével, mely során egyszerre alkalmazzuk a 3 különböző kezelést, eredményesen parazita mentesíthetők tóból érkező halak.
Kulcs szavak: Trichodinosis, gyrodactylosis, ichthyophthiriosis, parazitamentesítés, süllő.

Introduction. In recent years recirculation systems (RASs) have been gaining popularity quickly. In such fish-production systems, having a parasite- and pathogen-free environment, there is an absolute necessity for continuous production without serious losses (Timmons & Ebeling 2007). One way to achieve that is to keep the entire rearing cycle in a closed and intensive environment beginning with the breeding, on to the hatching and all the way down to the end product, the market-ready fish. This is a very obvious method, but unfortunately it is also very costly to provide all the necessary technology and conditions, not to mention the difficulties, costs and manpower-requirements of rearing larvae and young brood. For these reasons fish producers may need to opt for an open-lake alternative for pre-rearing of fish stocks that are later going to be moved to closed intensive systems. Such a chain of production can not even be contemplated without the knowledge and use of a proper disinfecting and deparasitizing procedure. If this issue is successfully handled than we get cheap, strong fingerlings or young of the year fish for further intensive rearing. Such fish transferred over from lakes may carry numerous pathogens and parasites, which may be too much for the immune system of the fish to hold back after the stress of mass-fishing and the change of environment. On top of this the increased population density is also advantageous for the spreading of pathogens and parasites. So it is clear that their propagation and spreading should be inhibited or prevented as soon as possible, and any fully developed parasites should be killed quickly as well.

Protection from pathogens and parasites is a complex task that begins with prevention. By providing the fish with optimal environmental factors (temperature, oxygen-level, feed, metabolite-free water) we also provide the ideal conditions for a strong immune-system to be built up in them. Correct handling, minimizing stress, protection from predators, pathogens and parasites are also crucial.

The interaction of the fish, its environment and the infecting agent will result in either the remaining of the healthy state of the animal or its infection. Thus the host-parasite relationship is a dynamic one. A few parasites are usually present on the fish, but it does not necessarily result in sickness or in any symptoms (MacMillan 1991). The importance of the environmental stress is illustrated by the fact that in catfish it is a common occurrence that 1-2 weeks after toxic methemoglobinemia, losses due to ectoparasite-infection will drastically increase (MacMillan 1991).

The immune system of fish is comprised of two parts: specific and non-specific. In case of a parasite-infection the non-specific immune-system will react first, then comes the specific immune-reaction, which usually takes more time. These two together are responsible for the developed protection (Buchmann et al 2001). For any effective treatment it is necessary to have an understanding of the biology of both the fish and the parasite, their effect on each other, and the role of the environment, plus the correlations between all these elements. So it follows that in a parasite-outbreak the possibilities of treatment and their success are influenced by many factors:

- species of fish, the age, the density and the condition of the population, the presence of other fish species (hosts)
- outside effects on the fish, stress, transportation, moving, mass-fishing
- water-properties (pH, hardness)
- temperature
- amount of dissolved oxygen
- floating matter concentration (organic and non-organic)
- length of treatment
- the feasible concentration and the stability of it over time

- in case of *Ichthyophthirius multifiliis* the binding surface necessary for cist-formation
- the circulation speed of water in closed systems, flow-speed of the water in open systems.

The goal of our experiment was to try a few of the existing deparasitizing procedures on a weakened population that had been kept in an over-wintering lake. The study was carried out on pike-perch, which is considered one of the weakest domestic species in terms of stress-resistance. We also had take heed that none of the methods or chemicals would be restricted in the EU or that they may not cause any damage to the bio-filter. After correlating data from the literature and experiences from fish-producers we chose the common table-salt (NaCl) for a low-dose long-term treatment and also for the short high-dose bath treatment, and the chemical known as DETOX-SA (manufacturer: ORPC; active ingredients: 4.5% peracetic acid, 10% acetic acid, and 20% hydrogen peroxide) which we used as a localized treatment while stopping the recirculating system for a short time. After 40 minutes the system can be restarted because that is how long it takes for the ingredients to exert the required effect and to break down.

Summary of the Literature

Ichthyophthiriosis. *Ichthyophtirius multifiliis* (Fouquet, 1876) is the parasite causing some of the most extensive losses in fresh-water fish production and it also difficult to defend against (Mehdizadeh Mood et al 2011). In lake fisheries it usually causes extensive damage during the spring in the stock weakened over the wintering period. If it gets into closed intensive systems, even less-susceptible species can suffer serious infestations and sometimes the total loss of the stock over a short period of time.

The sign of the infestation which can be seen even by the naked eye is the developed *Ichthyophthirius,* that is usually around 1 mm in diameter; this where the common name “white spot disease” comes from. In this stage the infestation is already quite advanced, treatment is difficult, and we need to be prepared for heavy losses. It is crucial to recognize the infestation in as early a stage as possible, so regular animal health control and attention to the environmental factors are a must. After significant stress an outbreak can be anticipated, so we can begin preventive action long before the symptoms even occur. The infestation can be recognized from the behavior of the fish: scratching themselves, jumping around a lot, heavy breathing (in case of serious gill-infestations the fish will “pump”). After first noticing such signs a quick and easy inspection under the microscope can confirm whether it is truly an *Ichthyophthirius* infestation that is the cause.

The species is a ciliate protozoa ectoparasite with three distinct stages in its life cycle (Figure 1).

After finding the host fish it will feed in the epithelium (on the surface of the body or the gills). Depending mostly on the environmental temperature the developed protozoa will cease feeding after a few days, leave the host animal, stick to some new surface and produce a cyst. There can be up to 500-1000 progeny (also called: “theronts”) formed in a single cyst. These when released can swim and find new prey.
The parasites released from the cysts will lose their attacking ability within two days of temperature drops to 20^{o}C. If they find a host they will burrow into the epithelium and begin feeding. In case of the death of the host all the parasites will leave the body shortly. The complete life cycle takes 4-5 days at temperatures between 24-26^{o}C, but may take as long as 40 days at 7^{o}C (Hoffmann 1978).

Ermolenko (1985) and Ewing et al (1988) posit that the entire life cycle may take place under the subephitelium as well.

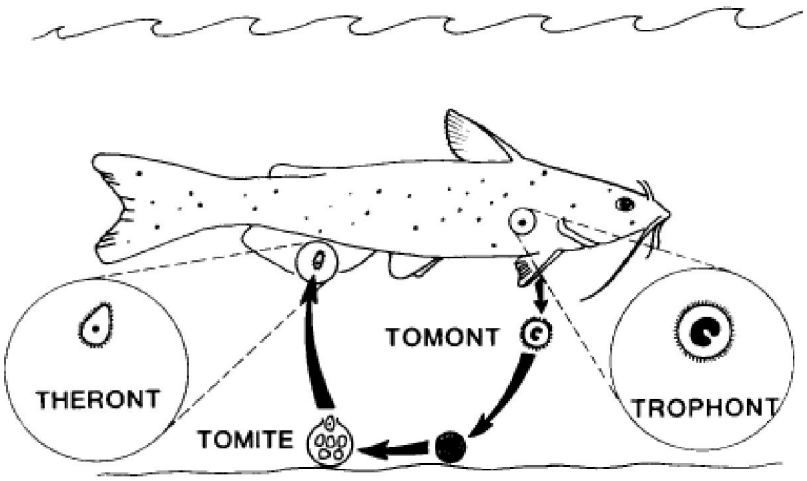

Figure 1. Life cycle of *Ichthyophthirius multifiliis.* Notes: TROPHONT: developed *Ichtyophtirius,* TOMONT: developed form, leaving the fish and sticking to a new surface forming a cyst, TOMITE: release the infectious form from the cyst, THERONT: swimming "attacking" form, actively seeking a host. Source: Ewing & Kocan (1992).

It was discovered nearly a century ago that those fish that didn't die due to the infection developed a resistance to *Ichthyophthyrius*. If they survive the infection then 3-4 weeks after first contacting the parasite an immunity may develop (Dickerson & Dawe 1995). Hines & Spira (1974) observed that carps (*Cyprinus carpio*) became immune to *Ichthyophthyrius* three weeks after surviving an infection. Rintamäki-Kinnunen et al (2005ab) observed in numerous experiments that 3-4 weeks after discovering the first specimen of *Ichthyophthyrius* the parasites began to disappear. These and similar findings are the driving force behind the efforts to find a vaccine (Burkart et al 1990; Dickerson & Dawe 1995). Ling et al (1993) achieved promising results by injecting material extracted directly from the parasites into the fish.

The malachite green was considered the most effective way of treatment against *Ichthyophthyrius multifiliis*, but due to its proven carcinogenic nature the use of this product was forbidden in fish production, both in the EU and in the USA. The restriction has been in effect since January 1st, 1998 in the EU and since October 1st, 2001 in Finland EC directive 90/676/EEC; article 14, regulation 2377/90/EEC). Cloramine-T is registered in the EU (98/8/EEC) and can be used in all of the countries of the EU to destroy the swimming forms by disinfecting the water, but it can not be used to treat the fish directly.

The search is on for possible replacement materials. The following externally applied chemicals have been tested: cloramine-T (Shinn et al 2001), potassium-permanganate (Straus & Griffin 2002), copper sulfate (Strauss 1993), sodium-percarbonate (Buchmann et al 2003), garlic extract (Buchmann et al 2003), peroxides and their mixtures, Desirox (Finnish Peroxides) (Rintamäki-Kinnunen et al 2005ab), (Meinelt et al 2009), potassium ferrate, silver nitrate (Farley & Heckmann 1980), toltrazuril proved effective (Schmahl et al 1989) but toxic to certain fish species (From et al 1992), and bronopol which is found in a product called Pyceze (Novartis Animal Vaccines Ltd.) (Shinn et al 2011).

Possibilities exist to defend against the parasite without using chemicals. One is to change the properties of the water, while the other is a type of mechanical protection. The properties of the water such as pH, saline-concentration, or the temperature can be an effective way in guarding against *Ichthyophthirius multifiliis*. Increasing the saline-concentration of the water has been used for a long time as a preventive defense against the parasite (Buza 1961). Utilizing this method in lake fisheries is not feasible, but it could be used in closed rearing systems. Though it doesn't destroy the parasite with such a high efficiency, but it helps in the regeneration of the epithelium, by increasing the production of mucus. According to Miron et al (2004) an adequate level of control can be achieved in the case of silver catfish (*Rhamdia quelen*) with 4g/L saline concentration.

After keeping the fish in such water for 45 days, the number of “white spots” decreased and the survival rate of the fish was 100%. This method could be successful especially with species that tolerate salt-water, and can be found in brackish water, such as the pike-perch (*Sander lucioperca*) used in our experiment.

Using UV-radiation (Gratzek et al 1983), or ozone to disinfect the water can be a solution for closed systems as well, provided that the circulation speed is set correctly. In our case we also used UV-disinfection to prevent cross-contamination of the tanks used in the experiment.

Trichodinosis. On the animals in our experiment we also found a *Trichodina* species in considerable numbers. These round ciliate parasites can also be identified with ease. On their adoral surface, within the sucking disk we will find a ring of cytoskeletal denticles, the number and shape of which is used to determine the exact species of the genus.

Numerous species are known, some of which reproduce specifically during the winter, others during the summer. Trichodinids are usually not species-specific. The larger species will live on the body surfaces of fish, the smaller species prefer the gills. They can achieve very high numbers and even cover the entire body surface of the fish, and by damaging the upper epithelium they can cause the death of the host. They generally cause the highest damages in the brood stock of herbivorous fish, coupled with other parasites they can cause significant losses, but brood stocks of catfish and carp can also suffer greatly from them (Molnár & Szakolczai 1980).

The first sign of infection is a change in the behavior of the fish. If the infection is located on the gills, the fish will become agitated, they begin to swarm in the more oxygenated layers of the water (usually up at the top), their movement may slow down considerably, even to the extent when one can easily catch them by hand. If the infection is located on the body surface, the fins will become torn, or ragged in appearance, and the large mass of dead epithelial cells will cause a grayish discoloration. They are counted as easily destroyable parasites (Molnár & Szakolczai 1980). Most treatments that are used against single-cell ectoparasites will be effective against Trichodinids as well (e.g.: 10-15-minute bath in 2.5% NaCl-solution, or 5-minute bath in 5% NaCl-solution).

Gyrodactylosis. The *Gyrodactylus* species are among the Monogenea flatworms. They are ectoparasites that develop directly, without any intermediate hosts. Under the microscope the one can clearly see within the translucent body of the worms all the developing progeny at different ages. They are strictly species-specific, which means that they will only choose a certain species of fish as host, or maybe occasionally a few closely related species.

Their body is translucent, the suction disc can be found on the tail end, with 16 small hooks around its edge, and two large hooks in the middle. The size and shape of these hooks are used in their precise classification. In lake fisheries only the species found on carps have significance (Molnár & Szakolcai 1980).

The infected fish become agitated, if the gills are infected, they will “pump” heavily. If the parasites infected the body surface of the fish, they will exhibit “scratching” activities.

Material and Method. We chose pike-perch as the subject species of our experiment. They were delivered from the wintering lakes of H&H Carpio Fish Farming Ltd. The transportation was done using plastic bags inflated with oxygen. The fish were delivered to fish-laboratory of the Georgikon Faculty of Agriculture of the University of Pannonia on January 9th, 2012. After weighing them on January 12th, 2012, we chose 492 specimen of 14.25 cm length ($\pm$ 0.45) and 20.57 g weight ($\pm$ 2.03) to be randomly distributed to a prepared 9-tank RAS (individual tanks had a capacity of 330 liter, the entire system had a capacity of 4,500 liters) and three separate round tanks (230 liters each) all located in the same room. We placed 41 fish in each of the 12 tanks. We provided aeration separately for each tank. The water circulation speed of the RAS was 6 liter/minute. To prevent cross contamination within the system we used two 18-watt UV-lamps (PHILIPS PL-18W), connected in series. In the individual tanks we filtered the water using sponge filters. During the experiment we daily exchanged 10% of the total capacity of each tank, providing fresh water to the fish. We measured the temperature, oxygen-concentration

and oxygen-saturation, and the saline-concentration (using conductivity) of the water every day. Illumination was provided by a 40-watt red spotlight per two tanks, because in our experience red light has a calming effect on pike-perch. The experiment ran for two weeks beginning with the first sampling, which took place on January 13th, 2012. After that, we took samples once a week and we did not feed the fish for the duration of the experiment.

We applied four treatments and replicated them three times:

- Control treatment, where we used no reagents at all. These three control groups where in the separate three tanks;
- 0.5 % continuous saline-solution (NaCl) treatment;
- 2% 10-minute-long saline (NaCl) bath on top of the continuous 0.5% saline treatment, which were applied immediately after the first and the second sampling;
- DETOX SA treatment on top of the continuous 0.5 % saline treatment: after the first sampling we applied 20 mL/m^3 treatments (as recommended by the manufacturer: ORPC), during which we stopped the recirculation for 40 minutes.

We reached the final saline-concentration in a four-day-long stretch starting from 0.2% (and going in 0.1% increments). This was done so that both the fish and the bacteria in the biological filter could get used to the higher concentration incrementally.

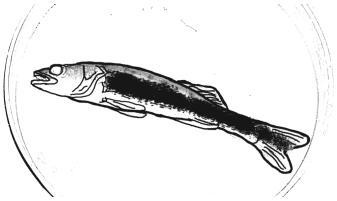

Figure 2. Sampled area (marked in black). Source: original.

After applying oil of clove to narcotize the fish, we took samples from five fish from each tank. The sampled area was on the side of the fish stretching from the stem of the pectoral fin to the end of the tail fin as illustrated on Figure 2 (the area marked in black). After the fish woke up they were returned to the same tank they were taken out from. We analyzed the resulting smears under a binocular optical microscope (Olympus, CH20BIMF200) using 10x, 100x, 400x magnifications. In the first step we classified the parasites found in the samples, then we counted them by species. We rated the infection of those parasites that occurred in larger mass, giving the following ratings: 0, low, medium and high. We organized the data using MS Excel, which we also used to create graphs.

Results and Discussion. During the experiment we encountered three groups of parasites (see Figure 3): *Trichodina sp., Gyrodactylus sp., Ichthyophthirius multifiliis*. In our results we will show the population changes of these parasites.

Figure 3. Photos of the parasites encountered (Original). (A) - *Trichodina sp.* (B) - *Gyrodactilus sp.* (C) - *Ichthyophthirius multifiliis*.

On Figure 4 we illustrated the population changes of *Trichodina sp.* At the time of the first analysis we observed an infection rate slightly higher than 'low'. This regressed slowly by the end of the second week in the control group, but didn't disappear completely while in the case of all the other treatments already by the 7th day we found no parasites of this genus.

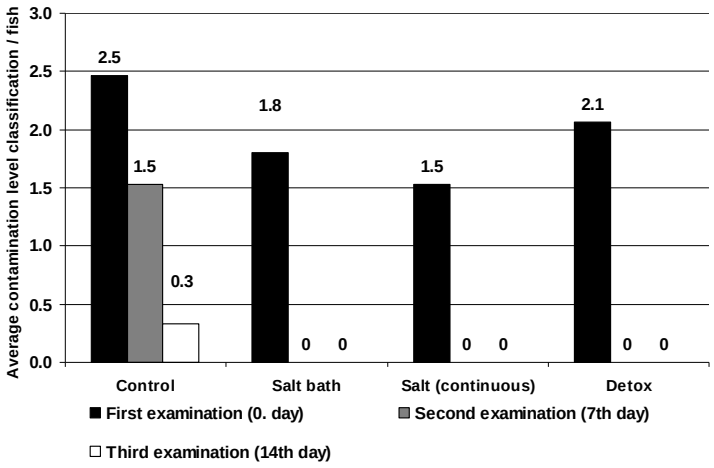

Figure 4. Changes in the population of *Trichodina sp*. during the course of the experiment.

On Figure 5 we illustrated the population changes of *Gyrodactilus sp*. By the 7th day of the experiment the average number of parasites per fish increased to 4.9 from 0.9 but by the 14th day it regressed back to 0.7. Both the short-time bath and the DETOX SA treatments were effective in our trial as the number of worms decreased drastically on the sampled area. The most effective was the DETOX SA treatment because on the 144th day of the experiment we found 0 parasites in the samples taken from this group. The continuous saline treatments proved ineffective because by the 14th day the parasite population grew to 30-times the original in the samples from this group.

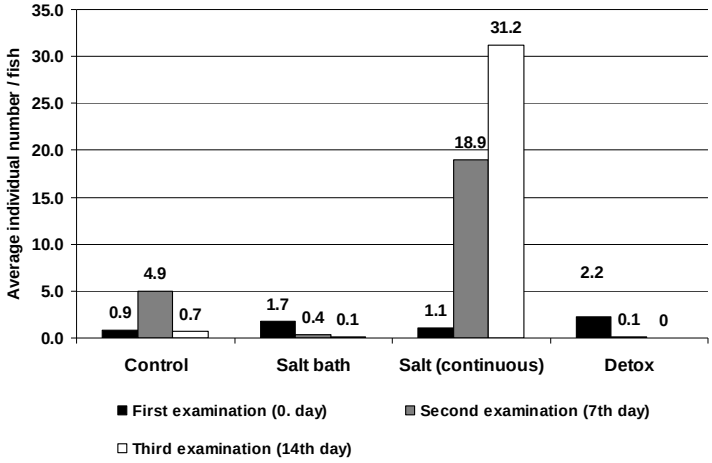

Figure 5. Changes in the population of *Gyrodactilus sp.* during the course of the experiment.

On Figure 6 we illustrated the population changes of *Ichthyophthirius multifiliis*. We found none of these parasites at the time of the first sampling in any of the groups of any of the treatments. We first encountered them in the control groups on the 7th day basically at an incidental rate (as it is shown on the graph as well). This very low presence turned into a very serious infection by the end of the second week. By this time we observed high numbers of theronts and trophonts alike. The parasite did not propagate in any of

the other groups, which can be accredited mainly to the continuous 0.5% saline treatment as the groups receiving this treatment alone were also free of infection.

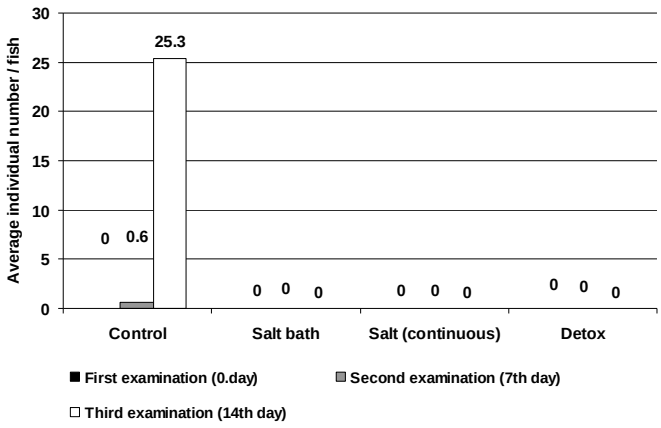

Figure 6. Changes in the population of *Ichthyophthirius multifiliis* during the course of the experiment.

On Figure 7 we illustrated the population changes of all three parasites. It is noticeable from the graph that the infection of the *Trichodina sp.* steadily diminished, the population of the *Gyrodactylus sp.* increased by the 7th day then decreased by the 14th, and the population of *Ichthyophthirius multifiliis* steadily grew. We can provide no confident explanation of this phenomenon but it is possible that the white spot disease simply outplaced the other two parasites from their environment.

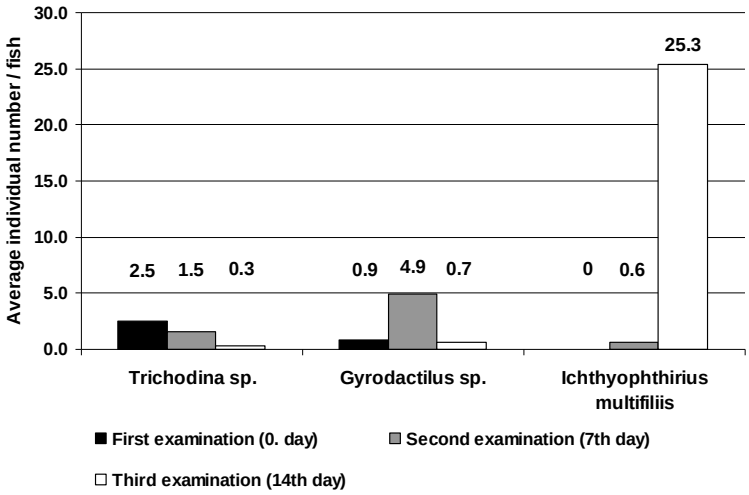

Figure 7. Parasite infection levels in the control groups.

During the course of our experiment we monitored the temperature, oxygen-saturation and saline-concentration (based on conductivity) of the water daily. The values of these parameters are given in Table 1. Significant changes did not occur in any of the tanks or at any time.

Table 1

Monitored water-parameters and their deviation during the course of the experiment

Parameter	Inflow to RAS tanks 1-9		Tank 10		Tank 11		Tank 12	
	mean	dev.	mean	dev.	mean	dev.	mean	dev.
Temperature ^{o}C	21.5	± 1.3	19.9	± 1.1	20.1	± 1.1	20.4	± 1.0
Oxygen mg/L	8.9	± 0.2	7.6	± 0.8	7.6	± 0.5	7.6	± 0.5
Oxygen-saturation (%)	99.9	± 1.6	83.8	± 4.1	82.5	± 4.3	83.9	± 4.4
Saline-conc. (ppt)	4.8	± 0.6	0.6	± 0.1	0.6	± 0.0	0.6	± 0.1

Conclusions. During our experiment we encountered the three groups of parasites that can be generally found in lake fisheries and which usually cause heavier infections after considerable stress to the fish stock or when the stock is weakened. Such effects often occur when fish are moved into an intensive system.

Based on our finding we can state that to repel the infection of the *Trichodina* sp. we need only to apply a prolonged 0.5% saline bath. The same bath however proved ineffective against the *Gyrodactilus* sp. which is probably due to the higher adaptation ability of the multi-cell organism, but by applying DETOX SA at 20 mL/m^3 we successfully broke the infection by the end of the second week. We were able to show that the 0.5% saline-concentration environment inhibits the propagation of the *Ichthyophthirius* which can lead to its use as a preventive measure. We consider the occurrences in the control groups to be very interesting observations in our trial. We can not give a definitive explanation why the populations of the other two parasites decreased so drastically when the white spots spread but we believe it is possible that we were witness to some phenomenon of population-dynamics.

Based on the experiment we can advise that using all three treatments during a 10-14-day-long period, fish stock from lakes can be successfully deparasitized.

From a practical standpoint it would be expedient to carry our further research to determine whether daily peroxide treatments are necessary, or is it enough to do them every other day, or maybe every third day. Also similar trials on other species of fish (e.g.: welsh catfish, *Silurus glanis*) would be useful.

References

Buchmann K., Sigh J., Nielsen C. V., Dalgaard M., 2001 Host responses against the fish parasitizing ciliate *Ichthyophthirius multifiliis.* Veterinary Parasitology 100:105–116.

Buchmann K., Jensen P. B., Kruse K. D., 2003 Effects of sodium percarbonate and garlic extract on *Ichthyophthirius multifiliis* theronts and tomocysts: in vitro experiments. North American Journal of Aquaculture 65:21–24.

Burkart M. A., Clark T. G., Dickeron H. W., 1990 Immunization of channel catfish, *Ictalurus punctatus* Rafinesque against *Ichthyophthirius multifiliis* (Fouquet): killed versus live vaccine. Journal of Fish Diseases 13:401–410.

Buza L., 1961 Sósfürdők a darakór leküzdésére [Salted water treatments against white spot disease]. Kisállattenyésztés, Budapest, 1.6., 23 p. [In Hungarian]

Dickerson H. W., Dawe D. L., 1995 *Ichthyophthirius multifiliis* and *Cryptocaryon irritans* (Phylum Ciliophora). In: Woo PTK (ed) Fish diseases and disorders, Vol 1. Protozoan and metazoan infections. Cab International, Wallingford, p.181–227.

Ermolenko A. V., 1985 On the reproduction of *Ichthyophthirius multifliis* (Ciliate, Ophryoglenidae). Parazitologiya 19:470-471.

Ewing M. S., Ewing S. A., Kocan K. M., 1988 *Ichthyophthirius* (Ciliophora): Population studies suggest reproduction in host epithelium. Journal of Protozoology 35:549-552.

Ewing M. S., Kocan K. M., 1992 Invasion and Development Strategies of *Ichthyophthirius multifiliis,* a Parasitic Ciliate of Fish. Parasitology Today, vol. 8, no. 6.
Farley O. G., Heckmann R., 1980 Attempts to control *Ichthyophthirius multifiliis* Fouquet (Ciliophora: Ophryoglenidae) by chemotherapy and electrotherapy. Journal of Fish Diseases 3:203–212.
From D. J., Karas N., Vordermeier T., 1992 Trials with toltrazuril against *Ichthyophthirius multifiliis* Fouquet (1876). Bulletin of the European Association of Fish Pathologists 12:137–138.
Gratzek J. B., Gilbert J. P., Lohr A. L., Shotts Jr. E. B., Brown J., 1983 Ultraviolet light control of *Ichthyophthirius multifiliis* Fouquet in a closed fish culture recirculation system. Journal of Fish Diseases 6:145–153.
Hines R. S., Spira D. T., 1974 Ichthyophthiriasis in the mirror carp *Cyprinus carpio* (L.). V. Acquired immunity. Journal of Fish Biology 6:373–378.
Ling K. H., Sin Y. M., Lam T. J., 1993 Protection of goldfish against some common ectoparasitic protozoans using *Ichthyophthirius multifiliis* and *Tetrahymena* for vaccination. Aquaculture 116:303–314.
MacMillan J. R., 1991 Biological factors impinging upon control of external protozoan fish parasites. Annual Review of Fish Diseases, pp. 119-131.
Meinelt T., Matzke S., Stüber A., Pietrock M., Wienke A., Mitchell A. J., Straus D. L., 2009 Toxicity of peracetic acid (PAA) to tomonts of *Ichthyophthirius multifiliis*. DAO 86: 51-56.
Mehdizadeh Mood S., Shohreh P., Sahandi J., 2011 A survey on ectoparasite fauna of cold water fish farms in Mazandaran Province, Iran. HVM Bioflux 3(3):246-251.
Miron D. S., Silva L. V. F., Golombieski J. I., Baldisserotto B., 2004 Efficacy of different salt (NaCl) concentrations in the treatment of *Ichthyophthirius multifiliis*-infected silver catfish, *Rhamdia quelen*, fingerlings. Journal of Applied Aquaculture 14:155–161.
Molnár K., Szakolczai J., 1980 [Fish Diseases]. Halbetegségek. Mezőgazdasági kiadó, Budapest [in Hungarian].
Rintamäki-Kinnunen P., Rahkonen M., Mannermaa-Keränen A. L., Suomalainen L. R., Mykrä H., Valtonen E. T., 2005a Treatment of ichthyophthiriasis after malachite green. I. Concrete tanks at salmonid farms. Diseases of Aquatic Organisms 64:69–76.
Rintamäki-Kinnunen P., Rahkonen M., Mykrä H., Valtonen E. T., 2005b Treatment of ichthyophthiriasis after malachite green. II. Earth ponds at salmonid farms. Diseases of Aquatic Organisms 66:15–20.
Schmahl G., Mehlhorn H., Taraschewski H., 1989 Treatment of fish parasites. 5. The effects of sym. triazinone (toltrazuril) on fish parasitic Ciliophora (*Ichthyophthirius multifiliis* Fouquet, 1876, *Apiosoma amoebea* Grenfell, 1884, *Trichodina* sp. Ehrenberg, 1831). European Journal of Protistology 24:152–161.
Shinn A. P., Wootten R., Somerville C., Conway D., 2001 Putting the squeeze on whitespot. Trout News 32:20–25.
Shinn A. P., Picon-Camacho S. M., Bron J.E., Conway, D., Yoon, G.H., Guo, F.C., Taylor, N.G.H., 2011 The anti-protozoal activity of bronopol on the key life-stages of *Ichthyophthirius multifiliis* Fouquet, 1876 (Ciliophora), Veterinary Parasitology 186(3-4):229-36. doi:10.1016/j.vetpar.2011.11.025
Straus D. L., 1993 Prevention of *Ichthyophthirius multifiliis* infestation in channel catfish fingerlings by copper sulphate treatment. Journal of Aquatic Animal Health 5:152–154.
Straus D. L., Griffin B. R., 2002 Efficacy of potassium permanganate in treating ichthyophthiriasis in channel catfish. Journal of Aquatic Animal Health 14:145–148.
Timmons M. B., Edeling J. M. 2007 Fish Health Management, Recirculating Aquaculture, ISBN 978-0-9712646-2-5 p. 619-620.

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