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Cpt. 14. Copepods vs. salmons: environmental treats for crustaceans or possible eco-

sustainable solutions?

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15.1. Introduction

Crustaceans may be objects of modern aquaculture productions, and in this case, they must be protected by various ectoparasites. However, they may represent, in their turn, threatening pests because some species are obligate parasites of fish (Ohtsuka et al. 2009). Conversely, multiple antiparasitic agents used in aquaculture practices may produce heavy impacts on natural populations of planktonic and benthic crustaceans (Bloodworth et al. 2019). In particular, the increasing use of drugs against parasitic crustaceans raised concerns about the potential impacts on non-target crustaceans (Langford et al. 2014), especially when they play key ecological roles, or they are valuable marketable species (Macken et al. 2015). In this chapter, we will analyze the effects of anti-parasitic agents and illustrate some new perspectives in the eco-sustainable treatment of fish infestations sustained by parasitic copepods.

Two key genera of sea lice are commonly found as parasites of salmonids in marine and brackish waters: *Lepeophtheirus* and *Caligus*. The so-called "Salmon louse" *Lepeophtheirus salmonis salmonis* (Krøyer 1837) is a copepod (family Caligidae) and it occurs in cold temperate waters of the northern hemisphere (Hamre et al. 2013; Skern-Mauritzen et al. 2021) as an ectoparasite of all

salmonid fish, so representing a major concern to Atlantic salmon (Salmo salar) aquaculture. In fact, the economic impacts of L. salmonis are estimated to induce a reduction from 6.2% to 8.7% of the productive value of salmonid fish (Costello 2009; Abolofia et al. 2017). Taking into account that Norway is the largest producer of salmon fish and that in 2018 a production of 1.28 million tons was declared (FAO, 2020), and including the productions of other nations providing relevant biomasses, the losses for the global salmon farming industry may be evaluated to be more than \$1.26 billion. When aquaculture farms are clustered in given regions, as in northern Europe and in Australia, they may further contribute to the outbreak of pathogens and to clinical diseases (Murray and Peeler 2005; Robertsen 2011). As well, along the east coasts of Canada and the USA, L. salmonis and C. elongatus represent emerging concerns both for aquacultural productions and natural conservation (Boxaspen 2006). In addition, environmental changes have increased the levels of infestation by L. salmonis and a continued increment of losses has been recorded in the last decade (Bjørndal and Tusvik 2019). Low-level infestations of sea lice may cause minimal effects on the host; however, high density of parasites and long-term infestations results in progressive worsening of skin damage and, finally, to the death of the host. For this reason, severe control regimes have been established to reduce the release of sea lice larvae from aquaculture facilities into the environment.

Their complete life cycle is well-known (Madinabeitia and Nagasawa 2011) and their taxonomical relationships have been described (Figure 1).

Figure 1. life cycle of *L. salmonis* with indication of the main planktonic and settled phases. Modified from (Schram and Others 1993).

The parasites hatch from typical egg strings carried by copepod females, to develop through three planktonic naupliar stages (nauplius 1 and 2, before the infective copepodid stage) nourished at the expense of their yolk sac (non-feeding). When a copepodid finds an adequate host, it attaches and develops through five further intermediate stages (Hamre et al. 2013) prior to reach the adult stage.

However, the number of stages between the infective copepodid and the adult may vary (Ho and Lin, 2004).

Seventeen species of Caligidae (Ohtsuka et al. 2009) are grouped into three genera, i.e., Caligus Müller 1785 (with 12 species), Lepeophtheirus von Nordmann 1832 (with 4 species) and Pseudocaligus A. Scott 1901 (containing a single species). All genera share the same number of stages in the free-living phase: as above mentioned, two nauplii and an infective copepodid stage, normally comprised within eight life stages (Hamre et al. 2013). The copepodid, following the attachment to the host, molts into the first chalimus stage. Chalimus stages (in variable number according to species and environmental conditions) are characterized by a frontal filament (Madinabeitia and Nagasawa 2011) that firmly secures their attachment to the host (Hamre et al. 2013). All known free-living copepods exhibit 6 post-nauplius stages, collectively referred to as copepodid stages. In fact, also Caligus spp. and most parasitic copepods exhibit 6 copepodid stages during their life cycle. The only genus, among copepods, exhibiting more than 6 copepodid stages is Lepeophtheirus (Hamre et al. 2013). Usually, L. salmonis exhibits a direct life-cycle completed over a single host. The mature female of a sea louse extrudes a pair of egg strings. The egg stage has a variable time span according to temperature and it may last from 17.5 days (at 5°C) to 5.5 days (at 15°C). Further, the planktonic nauplii stages above mentioned hatch directly into the water. Nauplii and copepodids are planktonic and phototactic and exhibit daily vertical migrations: they reach shallower depths during the day and sink at night. However, their ability to find their host is not lightdependent and interestingly, they may be found on various fish species, as a stop-gap measure, while seeking out their salmonid host (salmons, trouts and chars). They are also responsive to lowfrequency water accelerations, indicating the presence of a swimming fish: in this way they manage to find their hosts. Initial attachment of copepodids occurs on the fins of the fish or their scales. Initially, the copepodid clasps the host tissue. Further, it molts to the first sessile stage in the life cycle, up to the parasitic chalimus stage, starting to feed at the expense of the host fish. In fact, their planktonic stages live based on their own fat reserves and cannot feed until a host is found. The chalimus stage molts twice while it is attached to the fish, before becoming a pre-adult or mobile stage. At this stage, it is able to move around on the fish skin and can even swim around in the water column. The generation time for larvae is temperature-dependent too, and it varies from 2 to 4 weeks at 18 °C (Heuch et al. 2000). In contrast, adults are very longeval under all temperature conditions and the lifespan of an adult female, under laboratory conditions, was demonstrated to be more than 210 days (Schram and Others 1993). The adults feed on mucus, tissues and blood of their hosts, immediately inducing immunosuppression (Thorstad et al. 2015), followed by the appearance of skin sores and lower feeding efficiency.

The parasitic stages present on a host fish can be readily observed and enumerated. In contrast, the free-living planktonic stages can be identified only within zooplankton samples. This operation is quite challenging, given their low abundance, as compared to other planktonts (up to 0.70 ind. per cubic meter, according to (Skarðhamar et al. 2019). On the other end, it would be challenging as well to attempt fighting practices directed towards planktonic stages, due to their wide dispersal in the water column over large areas. Instead, it must be considered that aquaculture practices represent the primary sources of parasites because copepod larvae continuously escape from infested facilities located in northern Europe (Kristoffersen et al. 2018; Fjørtoft et al. 2019) and other world areas of intense production. In Europe, wild Atlantic salmon smolts migrate from rivers towards the Norwegian waters and they are often infected by L. salmonis. In fact, the increase of salmon aquaculture practices has been associated with the rise in L. salmonis infestations in nature and declines of wild salmonid populations (Heuch et al. 2011; Torrissen et al. 2013). The welfare concerns related to these observations prompted regulatory actions (Thorstad et al. 2015; Vollset et al. 2017). This suggests that disinfestation practices performed within aquaculture farms could not only improve the production efficiency and reduce the mortality of cultured fish but also reduce the lice loads in nature, so reducing their environmental impacts (Taranger et al. 2015).

15.2. Possible treatments in aquaculture and their environmental impacts

Salmon farming is commonly organized into two-phase production systems consisting of a landbased freshwater hatchery, followed by a marine cage site. Young salmons (from 1 to 2 yr after egg hatching) are transferred from the hatchery (as smolts) to sea cages, to increase their weight over a period variable from 18 to 24 months. As expected, sea lice represent a problem only during the second stage of the production cycle; recognising that transfers from land-based stages to the marine stage occur generally in spring or fall, these seasons represent the period of maximum risk of infestation. Various treatments were attempted for this purpose, with variable efficacy. To date, none of them appears to provide complete recovery. For example, Emamectin benzoate (EB, SLICE®) is an Avermectin chemotherapeutant (Horsberg 2012) and it is often administered to fish as an in-feed treatment lasting 7 days. Typical doses of EB in aquaculture feeds (Kuo et al. 2010) correspond to 50µg/kg fish biomass/day (Horsberg 2012; Benson et al. 2017). It has been used to treat infestations of sea lice *Lepeophtheirus salmonis* on farmed Atlantic salmon *Salmo salar* in the Bay of Fundy, New Brunswick, Canada, since 1999 (Jones et al. 2012).

On the whole, EB was demonstrated to be effective (Gustafson et al. 2006; Saksida et al. 2007) against *Lepeophtheirus salmonis* when applied to North American farmed Atlantic salmon (Armstrong et al. 2000). The time necessary to obtain the maximum effect of the treatments may be delayed in winter. In fact, Stone et al. (2000) observed that treatments applied during colder months reached the maximum effect later and the duration of the effect lasted for approximately 9 to 10 weeks, after the initiation of treatments (Lees, Baillie, et al. 2008). In contrast, reduced efficacy of EB has been reported in Scotland (Lees et al. 2008; Lees et al. 2008). Similarly, a reduced sensitivity of *Caligus rogercresseyi* to EB has been documented in Chile (Bravo et al. 2008), based on laboratory bioassays. The underlying causes of ineffective treatments are still undetermined (Jones et al. 2012). However, resistance to EB could be the primary cause for treatment failure, but also other reasons for reduced treatment efficacy (e.g., lower feed ingestion by fish in colder months, improper feed application or inappropriate concentration) may contribute to sub-therapeutic dosing and lead to failures. An additional issue is that EB is detected at considerable levels in sediments under coastal fish farm

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facilities (Cheng et al. 2020). It was estimated that only 5–15% of treated food remains uneaten, and carries EB in the environment (Chen et al. 2004; Langford et al. 2014). However, this must be summed to fish faces, that still contain EB for a period of 3–4 months post-treatment. Consequently, EB may enter the marine environments through various sources, even several months after the completion of treatments. Recent studies (Bloodworth et al. 2019) show that EB residues are more widely distributed in the benthic environment than previously thought, and have a strong impact on benthic communities. The degradation half-life of EB is longer than 120 days in marine sediments (according to the Authority and European Food Safety Authority, 2012) and its hydrophobic nature (National Center for Environmental Assessment, 2009) produces a long persistence in marine sediments around fish farm cages. This prompts a high risk of exposure for several benthic organisms. In addition, the bioactivity of EB is non-targeted: several crustaceans are subject to the same mode of action (Willis and Ling 2003). This represents a tremendous impact on several crustacean species (Veldhoen et al. 2012), given the large use of this compound for aquacultural activities all over the world.

For example, Park (2013) demonstrated a reduction in the abundance and the size at the harvest of the economically important prawn *Pandalus platyceros* immediately following treatments with EB, as compared to two months later (Park 2013). In a further study, Bloodworth et al. (2019) demonstrated an inverse exponential gradient of impacts in the response of benthic communities according to the distance from the cages, with the greatest impact observed under the cages, and widespread detection of EB (in 97% of samples) in the sampled sediments (Bloodworth et al. 2019). Remarkably, EB exhibits a single huge negative effect on crustacean abundance and diversity. The species richness of crustaceans is the environmental parameter most closely correlated to the impacts of EB. Small crustaceans, like copepods and amphipods, respond quite negatively to EB gradients (Hall-Spencer and Bamber 2007). The only crustacean apparently insensitive to EB accumulation in the sediments is the epifaunal hermit crab *Pagurus cuanensis* (Telfer et al. 2006). Differently, tube-building amphipods have their optima at moderate EB concentrations, probably due to their ability to

control the microenvironment in the tubes (de-la-Ossa-Carretero et al. 2016). Among amphipods, *Tanaopsis graciloides* and *Ampelisca tenuicornis* are able to switch between suspensions-feeding and deposit-feeding (Guerra-García and García-Gómez 2005; Ghodrati Shojaei et al. 2015) which potentially alters the vulnerability of these species to EB concentrations (Wilding et al. 2017). An additional demonstration of the effects of EB is given by the caprellid amphipod *Pariambus typicus*. In normal conditions, this species appears to respond positively to a moderate organic enrichment accumulated around fish farms (Fernandez-Gonzalez et al. 2013), probably due to its detritivore feeding habits. However, in presence of EB its abundance rapidly decreases, confirming the toxigenic effect of this drug. Finally, it is important to observe that bioturbation may accelerate the release of EB stored in marine sediments towards the water column, so increasing the impacts on planktonic crustaceans. However, low mobility taxa of crustaceans with a burrowing or a detritivore lifestyle are particularly vulnerable to EB and these findings demonstrate clear effects on all crustaceans, quite below the concentration of EB set by the current Environmental Quality Standards (EQS) at 0.763 µg/kg wet weight of sediment, at a distance of 100 m from the cages.

However, EB is not the only drug impacting natural populations of crustaceans. Another pesticide largely used in medicated feed for salmon for the control of sea lices is Diflubenzuron (DFB), a chitin synthesis inhibitor that interferes with the molting stages during the development of *Lepeophtheirus salmonis*. DFB spreading from salmon feeds may impact the natural populations of non-target crustaceans, such as the northern shrimp *Pandalus borealis*, an economically and ecologically important species in colder parts of the Atlantic (Bergström 2000; Moe et al. 2019). These shrimps accumulate DFB through the consumption of residues of medicated feeds for fish but also feeding on salmon faeces, as well as by ingesting other contaminated invertebrates (Langford et al. 2014). The recommended frequency of treatment with such Flubenzurones as DFB is every six months (Moe et al. 2019) and its application is commonly adopted in spring and autumn, when blooms of the parasitic copepods are expected. Overall, the harmful effect of DFB on the survival of shrimps (both adults and larvae) and on other benthic and planktonic crustaceans has been demonstrated by laboratory

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experiments (Bechmann et al. 2017; Bechmann et al. 2018). However, intensification of salmon aquaculture and of productive farms in general, led to increased use of several drugs and chemicals, including heavy metals, antibiotics and generic pesticides, most of which are detrimental to aquatic ecosystems and in particular to crustacean communities, due to demonstrated acute and chronic toxicity (Douet et al. 2009). For this reason, it is worth developing a new generation of anti-parasitic agents, based on physical methods (non-polluting) and natural substances (easily biodegradable and not accumulated in sediments and organisms). Physical methods as Catalytic Ozonation may be applied to the tanks for the culture of larvae and younger specimens, to reduce the presence of swimming larvae of parasites. Natural substances having a strong effect on adult sea lice are under investigation in various laboratories of the world, given the high economic impact of these parasites.

15.3. Drugs from nature: cyanobacteria vs. copepods

Cyanobacteria are an ancient group of organisms living in a wide range of marine and freshwater habitats, besides humid environments, and even in extreme conditions, as hot thermal waters and cold Antarctic environments. They are outstanding sources of secondary metabolites, often showing remarkable bioactivity. Besides oligopeptides (predominantly, cyclic peptides), other compound classes have been isolated from cyanobacteria, including terpenes and alkaloids. Although cyanobacteria have been more often studied as threatens for cultured fish, molluscs and other seafood (Landsberg 2002; Cazenave et al. 2005), due to their ability to intoxicate and kill, or to accumulate in tissues of fish and invertebrates, some of them might have a definite role in the eco-sustainable control of the sea lice and other fish parasites. In fact, besides the production of toxic metabolites (Mutalipassi et al. 2021), they are also enriched with several pharmacologically active compounds that have antibacterial (Volk and Furkert 2006; Malathi et al. 2014) and antifungal (Rath and Priyadarshani 2013) activities. Even anticancerous effects have been demonstrated (Gerwick et al. 1994; Mukund and Sivasubramanian 2014; El Semary and Fouda 2015). Interestingly some cyanobacteria exhibit antiplasmodial (Papendorf et al. 1998) and antiviral (Patterson et al. 1994; Abdo

et al. 2012; Riccio et al. 2020) activities. Other strains exhibit immunosuppressive activities (Koehn et al. 1992; Vijayakumar and Menakha 2015). Thus, due to a promising pharmaceutical value, a new perspective of using cyanobacteria and algae in the field of human and animal medicine has grown. Here we will briefly discuss how the active compounds present in the spent medium of a cyanobacteria culture demonstrated very interesting bioactivity against salmon parasites, so appearing as a promising solution for an eco-sustainable treatment of sea lices. In particular, we cultured a strain of *Halomicronema metazoicum*, isolated from leaves of the seagrass *Posidonia oceanica*, and we cultured it until active compounds were excreted in the culture medium. Further, we tested these compounds on sea lice to detect the possible effects at various concentrations.

15.4. Materials and Methods

15.4.1. Cyanobacteria cultures

A strain of *Halomicronema metazoicum* has been cultivated in continuous conditions at Stazione Zoologica Anton Dohrn. The strain has been isolated from *Posidonia oceanica* leaves and identified through a polyphasic approach using both morphological and molecular tools (Ruocco et al. 2018). The purity of the culture has been ascertained by means of SEM and molecular investigations and the absence of contaminating organisms has been demonstrated (Mutalipassi et al. 2019). Five 2L Erlenmeyer flasks were used to cultivate for 30 days 5 mats of *H. metazoicum*, each fresh pre-weighed and dosed at 5 (\pm 0.3) grams. The culture medium was prepared using filtered and sterilized natural seawater added with *f*/2 salts (Sigma-Aldrich, Merck KGaA, Darmstadt, Germany). The cyanobacteria were cultured in axenic conditions at a temperature of 22 °C with irradiance ranging from 130 to 160 µE and a photoperiod of 12/12 hours, at a salinity of 38 PSU. These culture conditions have been demonstrated to stimulate the production of bioactive compounds (Mutalipassi et al. 2019). At the end of the culturing period, spent media was collected and filtered using Stericup Filter Units (Sigma-Aldrich, Merck KGaA, Darmstadt, Germany). The collected spent medium was further

diluted using sterile seawater to obtain the desired concentrations for bioassays on sea lice and, in particular, it was diluted 1:5, 1:10, 1:100, 1:1000 and 1:10,000.

15.4.2. Bioassays

Toxicity tests were conducted on the sea lice Lepeophtheirus salmonis salmonis when they were in the phase of copepodite I-II. Copepodites were obtained from a local aquaculture centre in Sweden and they transferred to the testing laboratories in refrigerated containers. Before each experimental run, copepodites were checked under optical microscopy to check their vitality and then counted before being placed in the multiwell chambers. Only individuals that showed a healthy status were used for the activity bioassays. Copepodites represent the free-swimming infective stage and as above mentioned they do not need any feeding in this phase (Hamre et al. 2013). Two types of bioassays were performed, the first one using spent medium at various dilutions, the second one using 12 fractions at the same nominal concentrations of the raw spent medium. In the first experiment, five different dilutions of the cyanobacterial spent medium were tested (1:5, 1:10, 1:100, 1:1000, and 1:10,000 dilution of spent medium in water). Negative controls were prepared by adding fresh f/2medium to sterile seawater at concentrations corresponding to those of the above-mentioned treatments. Copepods (n=2) were incubated in a 24-well plate, using 8 replicated wells for each dose and treatment. Mortality and movements of sea lice were checked at time lang of 5 minutes, 60 minutes, 300 minutes, 24 hours, and 48 hours. The second experiment was carried out using the same protocol as the first, by exposing the sea lice to 12 fractions of the spent medium obtained by HPLC separations, at the same nominal concentrations of the medium above reported. The control replicates were obtained by adding diluted HPLC fractions of fresh medium.

15.4.3. Chemical fractionation

The GF/F filtered cyanobacterial culture supernatant and f/2 medium (as a control treatment) were adjusted to 1 % MeOH and subsequently passed through a preconditioned (100 % MeOH followed

by 1 % MeOH) Varian BondElut C18 SPE column. One litre of the cyanobacterial medium was extracted and the final extract was collected up to a volume of 500 µl. Columns were washed with 10 ml 1% MeOH and this aqueous eluate was collected in round-bottomed flasks (assumed 5x concentration *vs.* the original supernatant). Subsequently, the columns were eluted with 10ml of 100 % MeOH into two additional round-bottomed flasks. Each HPLC run was performed with 10 uL of the cyanobacterial extract which resulted in 12 fractions. All fractions were tested on *Lepeophtheirus salmonis salmonis* at nominal concentrations corresponding to the ratios of the spent medium in seawater, as above reported for the bioassays on crude medium, *i.e.*, corresponding to ratios of 1:5, 1:10, 1:100 and 1:10,000 of spent medium:water.

15.4.4. Statistical analysis

Data deriving from various replicates were organized as means with standard deviations for each set of measurements. The differences among various concentrations and controls were analyzed using two-way ANOVA. Dunnett's multiple comparisons posthoc tests were applied to check differences against negative controls of each treatment at each time. Results from spent medium and fractions tests were analyzed by two-way ANOVA with Tukey's multiple comparisons posthoc tests to evaluate the significance of differences between replicates at the same dilution and at the same experimental time. Data were tested for normality by the Shapiro-Wilk normality test. Graphs and statistical analyses were computed using GraphPad Prism version 7.00 for Windows (GraphPad Software, La Jolla California USA).

15.5. Results and conclusive remarks

The replicated bioassays performed using crude spent medium produced a linear pattern of responses in sea lice, according to dilutions (Figure 2). In treatments added with the highest concentration (1:5, 1:10, 1:100) of the spent medium, the survival was null, yet at the first time-lag (5 minutes), indicating the highest toxicity of the cyanobacterial compounds (Figures 2A, B, C). At a dilution of 1:1000, the survival was 100% at the first time lag (5 minutes), then started to decrease, indicating time-related toxicity. In fact, at the second time lag (60 minutes) the survival was 87.5 (\pm 13.4) and at the third time lag (300 minutes) it was 75.0 (\pm 18.9). After 24 hours, however, sea lice were all dead (Figure 2D). In the treatment at a concentration of 1:10,000, no mortality was exhibited until the fifth time lag (48 hours) were survival decreased to 18.75 (\pm 25.88). Statistical analyses confirmed the significance of differences above reported for a dilution of 1:1000, at time lags 2, 3, 4, 5 (two-way ANOVA, p<0.0001), and for a dilution of 1:10,000, at the time lag 5 (p<0.0001).

Figure 2. Survival of *Lepeophtheirus salmonis salmonis* during the bioassays conducted at various concentrations of *H. metazoicum* spent medium (A-E), from 1/5 to 1:10,000

These results indicate a specific effect dependent on time and doses, of some active compounds present in the spent medium of *H. metazoicum*. It also demonstrated the concentration of 1:1000 as the lowest threshold of activity, able to kill all sea lice during the first 24 hours of treatments. During this time, these concentrations may be toxic also to young fish. However, it must be considered that the compound is easily stored in the body of sea lice. For this reason, shorter treatments (less than 1 hour) may be sufficient to produce effects within 24 hours, when sea lice are transferred in clean water along with their hosts. This study demonstrates that the treatment with cyanobacterial active compounds may be a very promising and eco-sustainable solution to treat the infested fish, by means of short baths in water added with a spent medium of *H. metazoicum*.

To further characterize the nature of the active compound/s present in the spent medium, bioassays were performed using HPLC fractions of the spent medium, at the same nominal concentrations of the previous bioassays (*i.e.*, at concentrations corresponding to 1:5, 1:10, 1:100, 1:1000 and 1:10,000 of the spent medium from which they were extracted). These tests confirmed the total absence of

toxicity at nominal concentrations of up to 1:100. The fractions tested at a nominal concentration of 1:1000 consistently demonstrated activity on the survival of sea lice, in agreement with the test performed on the whole spent medium (Figure 3).

Figure 3. Survival tests performed at a concentration of fractions corresponding to a dilution of 1:1000 of the original spent medium. Only the active fractions have been reported, for brevity, since all the other fractions exhibited no effect on the survival of *Lepeophtheirus salmonis salmonis*, even at maximum doses.

In particular, three fractions had a toxic effect on sea lice: fraction n° 8 demonstrated the maximum toxicity, leading to the death of all the tested individuals at the third time lag (300 minutes). Fraction 9 demonstrated intermediate toxicity, by exhibiting survival of 83.3 (\pm 12.9) at the third time lag (300 minutes), and correspondingly, 58.3 (\pm 12.9) at the fourth time lag (24 hours), and 54.2 (\pm 10.2) at the fifth time lag. The differences *vs.* negative controls were statistically significant (two-way ANOVA, p<0.0001 at the time lags 4 and 5). In contrast, fraction 7 was the least toxic (two-way ANOVA, p<0.0001), exhibiting significant differences among controls only at the fourth time lag (24 hours) and the fifth (48 hours).

On the whole, these results indicate that the compound was maximally collected in fraction 8 and it was a partially polar compound of small molecular weight. The active concentration of 1:1000 may be toxic both for sea lice and young fish, after 24 hours of exposition. However, sea lice quickly accumulate the compound in their body, while fish are able to detoxify and excrete the toxic compound. For this reason, shorter treatments (baths of 45 minutes, followed by a total change of water) were demonstrated to be sufficient to obtain complete detachment of sea lice from the body of their hosts and dead of sea lice sinking on the bottom after some hours. The compound appears to be enough stable in natural conditions, even at higher temperatures, but it is quickly degraded in the natural environment. Since it is active even after short baths, treatment tanks could be set to assure total recovery of cultured fish in a few hours, prior to being returned to the cages.

Such an environmental-free and eco-sustainable treatment is very promising, permitting to reduce the impacts of parasites on cultured fish and, in addition, reducing the possibilities for the parasites to invade the surrounding environments, impacting the natural populations of salmons.

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15.7. References

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