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The effect of chemotherapeutic drugs used to control sea lice on the hatching viability of egg strings from *Caligus rogercresseyi*

Sandra Bravo [a,*], María T. Silva [b], Celia Agusti [c], Karla Sambra [a], Tor E. Horsberg [c]

[a] Universidad Austral de Chile, Puerto Montt, Chile
[b] Universidad San Sebastian, Puerto Montt, Chile
[c] School of Veterinary Science, Sea Lice Research Centre, Norwegian University of Life Sciences, Oslo, Norway

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ABSTRACT

The effect of four treatments used in the control of sea lice on the fecundity rate of *Caligus rogercresseyi* was assessed. Samples of gravid females were collected from farmed rainbow trout in a site located in southern Chile (43°8′S, 73°35′W). Gravid females were exposed to 0.4 and 2 ppb azamethiphos, 0.2 and 1 ppb deltamethrin, 100 and 500 ppb emamectin benzoate, and 42 and 336 ppm hydrogen peroxide in separate baths. After 24 h of exposure at controlled temperature and photoperiod, the mortality of females was evaluated and the egg strings removed. Egg strings were incubated in fresh filtered seawater at 12 °C and were daily evaluated for a period of 7 days. In the deltamethrin group, a hatching of 67% and 61% of the egg strings was recorded for on the fourth day in the low and the high concentration of deltamethrin, respectively. In the emamectin benzoate group, 43% and 42% of egg strings hatched at the low and high concentrations, respectively, while the corresponding figures for azamethiphos were 44% and 50%, and for hydrogen peroxide 36% and 50%. The emerged nauplii were observed to be inactive in all exposed groups. In the control group, hatching began on the second day, and the first copepodids were seen on day seven. Even though the exposures were done for 24 h while ordinary bath treatments last for 30–60 min, the results indicate that treatments with antiparasitic products to control sea lice could have a detrimental effect on the maturation and hatching of exposed eggs strings, as well as over the survival of exposed larvae of *C. rogercresseyi*.

1. Introduction

Sea lice is the most severe threat affecting the salmon industry worldwide. In the northern hemisphere, the main louse species affecting Atlantic salmon (*Salmo salar*) is *Lepeophtheirus salmonis*, while in the southern hemisphere *Caligus rogercresseyi* is the louse species affecting Atlantic salmon and rainbow trout (*Onchorhynchus mykiss*), the two more susceptible salmonid species to sea lice in Chile (Bravo, 2003; Pino-Marambio et al., 2007). The life cycle of *C. rogercresseyi* includes eight developmental stages: two planktonic nauplii, an infective copepodid, four attached chalimus stages and an adult stage, without pre-adult stages. Free living nauplii larvae are released from the hatching eggs while the paired egg strings are still attached to the adult female. Copepodids and adult lice can survive up to 7 days free in seawater without a host (Bravo, 2010a), enough time to find a salmonid host in the densely farmed waters of southern Chile.

In contrast to Norway, where several non-chemical methods are used increasingly to remove or reduce the number of lice attached to farmed fish (Aaen et al., 2015), chemicals have been the main tools to combat sea lice so far in Chile. At the beginning of the Chilean salmon farming, organophosphates applied by bath were used (Reyes and Bravo, 1983), followed by oral treatments (Bravo, 2003; Bravo et al., 2008). Currently the organophosphate azamethiphos and two pyrethroids, cypermethrin and deltamethrin, are the three main products used as bath treatments. Hydrogen peroxide was mainly used during 2007 as a bath treatment, and it was also used at low concentrations to detach lice before harvesting during transportation from the farm to the slaughter place in well-boats.

As with *L. salmonis*, there is an increasing concern about the risk of resistance development in *C. rogercresseyi* towards the medicinal products used for its control. Evidence of resistance towards emamectin benzoate was reported in Chile (Bravo et al., 2008, 2010a), and treatment failures and resistance development were recorded for pyrethroids (Bravo, 2010b; Helgesen et al., 2014). In the case of azamethiphos and hydrogen peroxide, preliminar data based on 24-h bioassays suggest that *C. rogercresseyi* has not reduced sensitivity towards these compounds in Chile (Agusti and Bravo personal observation, 2014). However, reduced sensitivity towards hydrogen peroxide was recorded for *L. salmonis* in Scotland (Treasurer et al., 2000) and recently in Norway (Helgesen et al., 2015). In the case of azamethiphos, resistance has been reported in Norway for *L. salmonis* as well (Grøntvedt et al., 2014).

* Corresponding author. Tel.: +56 65 2277121; fax: +56 65 2255833.
E-mail address: sbravo@uach.cl (S. Bravo).

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Because resistance is hereditary (Bravo et al., 2010a; Denholm et al., 2002; Helgesen et al., 2015) and because it is equally essential to inactivate or kill the progeny as well as the progenitors, it is important to know the effect on the hatching ability of *C. rogercresseyi* egg strings exposed to the chemotherapeutic products. Toovey and Lyndon (2000) reported that hydrogen peroxide, dichlorvos and cypermethrin significantly reduce the hatch rate in *L. salmonis* and also the development of the larvae to nauplius and copepodid stages. In addition, Aaen et al. (2014) reported that hydrogen peroxide significantly affects hatching of egg strings and development of *L. salmonis* larvae. Thus, the aim of this study was to assess, under laboratory conditions, the hatching ability of egg strings collected from *C. rogercresseyi* gravid females exposed to two concentrations of azamethiphos, deltamethrin, emamectin benzoate and hydrogen peroxide.

2. Methodology

2.1. Lice collection

Samples of gravid females were collected in the autumn of 2014 from farmed rainbow trout in a site located in southern Chile, Region X (43°8′S, 73°35′W). Lice were carefully removed from infested rainbow trout anaesthetized with benzocaine (10% in ethanol, 1ml/L) and transported live in filtered, cooled and aerated seawater to the laboratory at the Aquaculture Institute of the Universidad Austral de Chile in Puerto Montt. The studies were initiated within six hours post collection.

2.2. Sensitivity assessment

The 24-h bioassays were performed according to the protocol described by Helgesen and Horsberg (2013). Twelve gravid females with egg strings were randomly distributed in each of nine one-litre glass bottles filled with filtered seawater. Gravid females were exposed to 0.4 and 2 ppb (low and high dose respectively) of azamethiphos (Sigma-Aldrich); 0.2 and 1 ppb deltamethrin (AMX, Pharmaq); 100 and 500 ppb emamectin benzoate (Sigma-Aldrich), and 42 and 336 ppm hydrogen peroxide (Veterquímica). The working solution for each product was prepared on the same day. One control group was established with 12 gravid females maintained in fresh filtered seawater. Bottles with gravid female lice were supplied with aeration, and kept for 24 h in an incubator at a temperature of 12 °C and a photoperiod of 12 h light and 12 h darkness.

Mortality of lice was evaluated immediately after the 24-h exposure, following the criteria proposed by Sevatdal and Horsberg (2003), that is, *dead*: no movements; *moribund*: not capable of attaching to the wall of the glass bottle using the flat body as a "sucking disc," but movements of extremities or internal organs could still be observed; *live*: attached to the walls of the glass bottle or active swimming behavior.

2.3. Hatching assessment

After the 24-h exposure, the detached egg strings were collected from each bottle and transferred to glass containers with 100 ml of fresh filtered seawater, labeled for each concentration and product. Egg strings still attached to the female were carefully removed with a scalpel and transferred to the glass containers. The condition of the egg strings was evaluated immediately before they were transferred to fresh seawater and classified as unpigmented (immature), pigmented (mature) and under hatching. Twenty egg strings, including pigmented, unpigmented and under hatching, from each product and concentration (low and high dose) were kept at 12 °C. Maturation and hatching evolution of the egg strings was evaluated daily for a period of 7 days (Fig. 1a–e). Results were expressed as the percentage of hatched egg strings. Abortion was defined as the nauplii being dead at hatching (Fig. 4). Nauplii emerging during the 24-h exposure to each treatment were collected with a plankton net and the survival was subjectively evaluated.

2.4. Statistics

The non-parametric Mann–Whitney *U*-test was used to assess differences in the hatching between doses (low and high) for each treatment. All observation time points in the period of study were included. The same was done for maturation (development of pigmentation) of the unpigmented egg strings. The *Z* test for significant differences of proportion was used to assess the pairwise differences on the hatching, as well as for maturation of unpigmented eggs strings between treatments at the end of the period of study. Differences were considered significant when $p < 0.05$.

3. Results

3.1. Adult lice sensitivity

The mortality rate of gravid females of *C. rogercresseyi* exposed 24 h to the low and high concentration of azamethiphos (AZA), deltamethrin (DTM) and hydrogen peroxide (PER) was 100%, whereas for emamectin benzoate (EMB) the mortality rate was 10% at the low concentration and 35% at the high. No parasites died in the control group.

3.2. Hatching success and larvae survival

In the control group, hatching commenced during the second day and continued during the next days. The onset of hatching was correlated with the degree of pigmentation of the egg strings. All the egg strings had hatched at day six (Fig. 1a), and the first copepodids were seen on day seven.

The eggs exposed to the four medicinal products showed diverse degree of hatching and egg development. All nauplius-I emerged from the eggs during the exposure of the gravid females were found dead at the end of the 24-h exposure. The subjective evaluation of the number of nauplii emerging in each group showed that the highest proportion was recorded for azamethiphos (2 ppb) and deltamethrin (1 ppb), moderate proportion was recorded for azamethiphos (0.4 ppb), while the lowest proportion was recorded for emamectin benzoate (500 ppb) and hydrogen peroxide (336 ppm).

During the 7 days of the egg incubation in fresh seawater, hatching occurred in every group, but all eggs were aborted in every hatch in the groups exposed to treatments, with the exception of the low azamethiphos dose. The first day after exposure, the highest hatching rate occurred in the egg strings exposed to the high azamethiphos concentration, followed by those exposed to the high concentration of hydrogen peroxide (Fig. 2a and b).

3.2.1. Azamethiphos

Hatching started the first day, both at the low and the high dose. At the low dose, the maximum hatching abortion (44%) was recorded on day five, while for the high dose, the maximum hatching abortion (50%) was recorded the second day. Live nauplius-I were recorded only at the low concentration of azamethiphos during the first day of incubation in fresh seawater, but they showed slower movements compared with nauplius-I from the control group and died during that same day, not developing into nauplius-II. Therefore, no viable copepodides developed from the hatched eggs. Unpigmented egg strings exposed to the low dose remained unpigmented from the day three (31% of the total number of unpigmented egg strings). Egg strings exposed to the high dose remained unpigmented from day five (21%) (Fig. 1b).

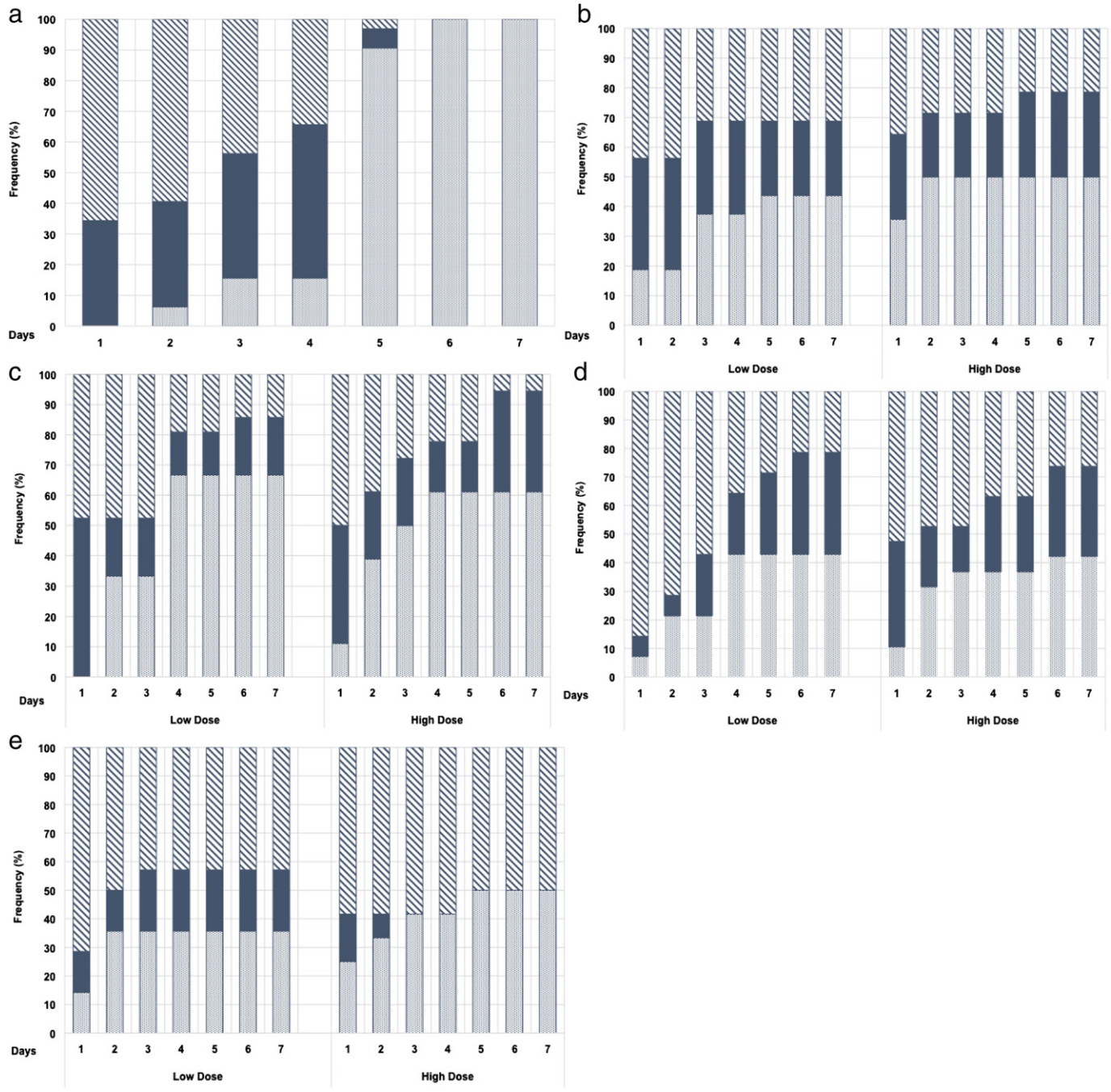

Fig. 1. (a) Development of the maturation of the egg strings of the control group through the period of incubation. Top column corresponds to unpigmented egg strings; medium column (black) to pigmented egg strings and lower column to hatched egg strings. (b) Development of the maturation of the egg strings exposed to low (left) and high (right) dose of azamethiphos through the period of incubation in fresh seawater. (c) Development of the maturation of the egg strings exposed to low (left) and high (right) dose of deltamethrin through the period of incubation. (d) Development of the maturation of the egg strings exposed to low (left) and high (right) dose of emamectin benzoate through the period of incubation. (e) Development of the maturation of the egg strings exposed to low (left) and high (right) dose of hydrogen peroxide through the period of incubation.

3.2.2. Deltamethrin

Hatching on the first day was only recorded at the high dose. For both doses, the maximum hatching abortion was recorded on day four (67% for the low dose and 61% for the high dose). No live larvae were recorded from the hatched eggs exposed to this treatment. From day six, the unpigmented egg strings remained unpigmented, 14% at the low dose and 6% at the high (Fig. 1c).

3.2.3. Emamectin benzoate

Hatching was recorded from the first day, both at the low and the high dose. At the low dose, the maximum hatching abortion (43%) was recorded on day four, while for the high dose, the maximum (42%) was recorded on day six. Again, no live larvae were recorded from the hatched eggs. From day six, no further pigmentation of unpigmented egg strings was noted. At the low dose, 21% remained unpigmented, while the corresponding number for the high dose was 26% (Fig. 1d).

3.2.4. Hydrogen peroxide

Hatching was recorded from the first day, both for the low and the high dose. At the low dose, the maximum hatching abortion (36%) was reached the second day, while for the high dose, the maximum

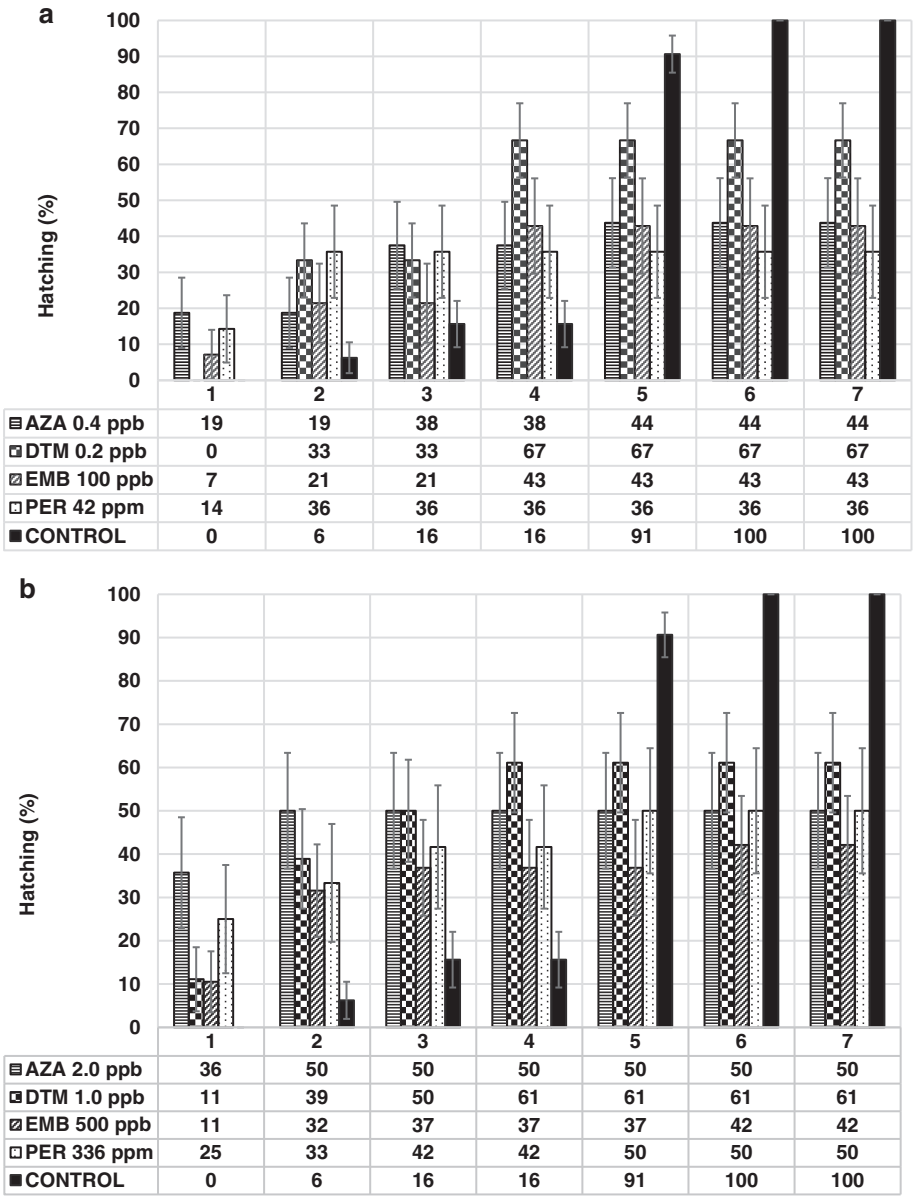

Fig. 2. (a) Cumulative hatch rate (mean ± SE) of the egg strings exposed to low concentration of azamethiphos (AZA), deltamethrin (DTM), emamectin benzoate (EMB) and hydrogen peroxide (PER) in comparison with the untreated control egg strings. (b) Cumulative hatch rate (mean ± SE) of the egg strings exposed to high concentrations of azamethiphos (AZA), deltamethrin (DTM), emamectin benzoate (EMB) and hydrogen peroxide (PER) in comparison with the control egg strings.

rate (50%) was recorded on day five. No live nauplius was seen at any time point of the study. From day three (low dose) or five (high dose), no more pigmentation of unpigmented egg strings could be seen. The remaining percentages of unpigmented strings were 43% (low dose) and 50% (high dose) (Fig. 1e).

No significant differences in the hatching rate between the high and the low concentrations were recorded during the observation period in egg strings exposed to deltamethrin ($p = 0.745$), emamectin benzoate ($p = 0.745$) and hydrogen peroxide ($p = 0.182$). A significant difference was only recorded for azamethiphos ($p = 0.019$) (Fig. 3a). For the maturation of unpigmented egg strings, significant differences were also recorded only for azamethiphos ($p = 0.021$) (Fig. 3b).

At the end of the study period, significant differences in the hatching abortion were only recorded between deltamethrin and hydrogen peroxide at the low doses ($p = 0.030$) (Table 1), where the highest hatching rate was recorded in egg strings exposed to 0.2 ppb deltamethrin (67%) and the lowest for egg strings exposed to 42 ppm hydrogen peroxide (36%) (Fig. 2a). No significant differences were recorded between treatments at high doses ($p > 0.1$) (Table 1), but the highest hatching abortion was also reported for deltamethrin (61%) while the lowest was recorded for emamectin benzoate (42%) (Fig. 2b). Significant differences in the proportion of unpigmented egg strings becoming pigmented during the observation period were at low doses only recorded between deltamethrin and hydrogen peroxide ($p = 0.031$). For the high doses, differences were recorded between deltamethrin and hydrogen peroxide ($p = 0.002$), deltamethrin and emamectin benzoate ($p = 0.035$), and deltamethrin and azamethiphos ($p = 0.041$) (Table 1).

4. Discussion

To our knowledge, this is the first study aiming to analyze the *C. rogercresseyi* hatching viability of egg strings exposed to chemotherapeutic products used for its control. The experiments were performed on parasites detached from the fish, with an exposure time of 24 h and, with exception of the oral agent emamectin benzoate, at

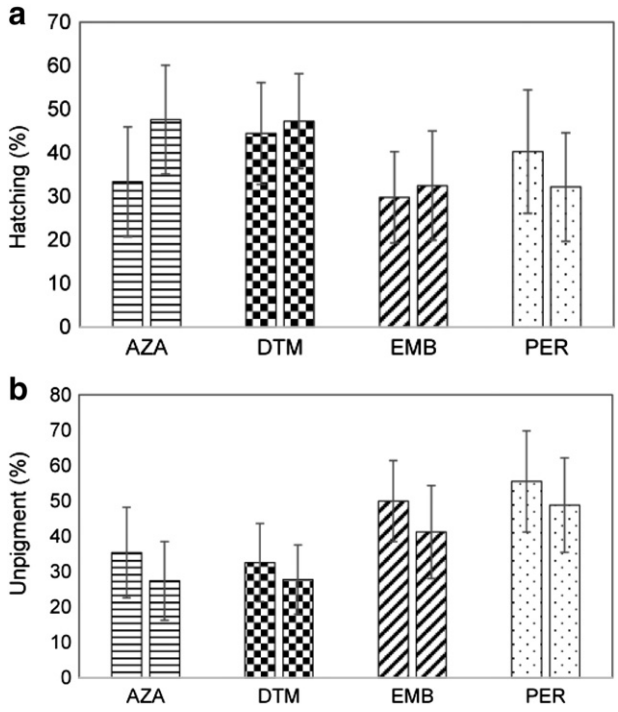

Fig. 3. (a) Comparison of the mean hatch rate (mean ± SE) of the egg strings exposed to low concentrations (left) and high concentration (right) of azamethiphos (AZA), deltamethrin (DTM), emamectin benzoate (EMB) and hydrogen peroxide (PER) through the period of study. (b) Comparison of the unpigmented rate (mean ± SE) of the egg strings exposed to low concentrations (left) and high concentration (right) of azamethiphos (AZA), deltamethrin (DTM), emamectin benzoate (EMB) and hydrogen peroxide (PER) through the period of study.

concentrations far below concentrations used in clinical treatments. The exposure time and concentrations were selected because they had been demonstrated to separate well between parasites with full sensitivity and reduced sensitivity towards the different treatments (Helgesen and Horsberg, 2013; Helgesen et al., 2014). Although the experimental conditions differed from clinical treatments, the results pointed towards a significant additional effect of sea lice treatments.

Abortion, i.e., dead nauplii at hatching, was recorded in most of the exposed egg strings to the two concentrations of azamethiphos, deltamethrin, emamectin benzoate and hydrogen peroxide, and when viable nauplii hatched, such as with azamethiphos at low dose (0.4 ppb), the larvae did not developed to copepodids.

In the control group, hatching started at the second day post incubation, 100% of the egg strings hatched on the day six and the first viable copepodids appeared at day seven. The egg strings exposed to azamethiphos, hydrogen peroxide and emamectin benzoate hatched on the first day, the highest rate was for egg strings exposed to azamethiphos. A higher hatching abortion rate was recorded for the high dose in comparison with the low dose on day one after exposure for all compounds (Fig. 2a and b). However, significant differences in the hatching abortion between the low and high concentration were only recorded for the azamethiphos treatment. The highest cumulative hatching abortion was recorded for deltamethrin, both in the high and low concentration groups.

Regarding development of the eggs, the percentage of immature egg strings remained high through the whole study period when gravid females were exposed to hydrogen peroxide. This could indicate a detrimental effect of the drug also on the maturation of the eggs. A similar effect, but at lower degree, was observed with the exposition to azamethiphos. Unpigmented eggs exposed to deltamethrin and emamectin benzoate developed pigmentation, but at lower rate than in the control group.

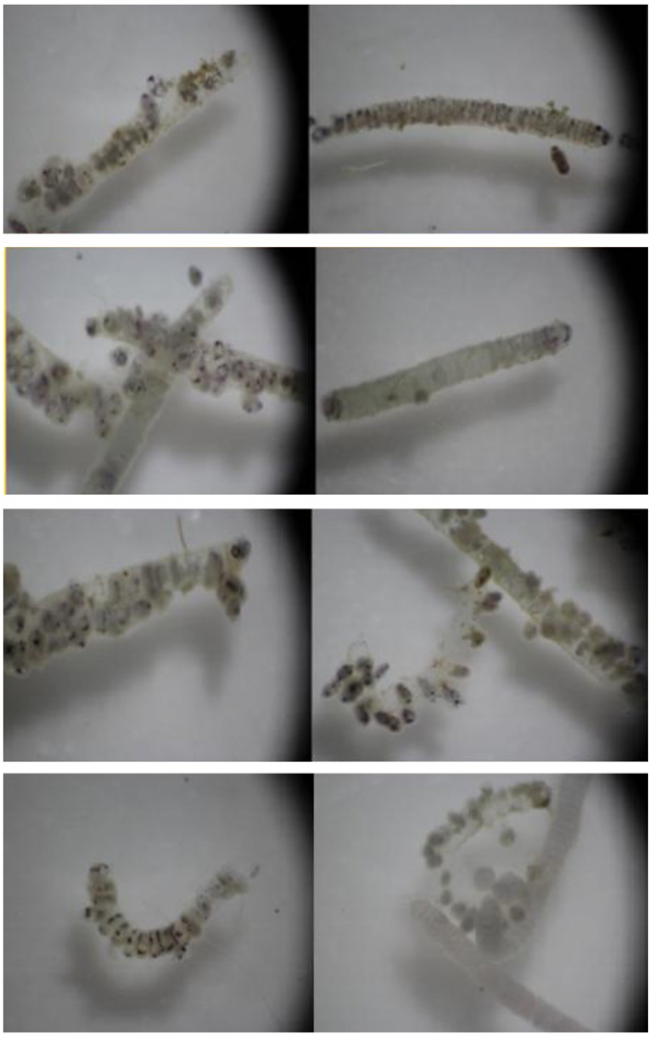

Fig. 4. Abortion in egg strings exposed to low (left) and high (right) concentration of deltamethrin (DTM), emamectin benzoate (EMB), azamethiphos (AZA) and hydrogen peroxide (PER).

The results obtained in this study are in accordance with results reported by Toovey and Lyndon (2000). Under ordinary fish farm conditions, they collected ovigerous females of *L. salmonis* shortly after treatments with hydrogen peroxide, dichlorvos and cypermethrin. As controls, gravid females were again collected 9 weeks post treatment. For all treatments, a significant reduction of hatching was recorded in egg strings from gravid females collected shortly after the treatments compared to the hatching from the control parasites. Reduced production of larvae in *L. salmonis* after dichlorvos treatment was also reported by Bron et al. (1993). Studies *in vitro* carried out by McAndrew et al. (1998) with hydrogen peroxide showed a detrimental effect on the egg viability, as well as on the chalimus development in *L. salmonis.* They also reported that egg strings exposed to this product showed altered pigmentation associated with reduced viability. Similar results were recorded by Aaen et al. (2014) for *L. salmonis* under laboratory conditions, where a 30-min exposure to hydrogen peroxide concentrations ranging from 470 to 2000 ppm were demonstrated to prevent the development of copepodids.

The gravid females that carried the egg strings died when they were exposed to the two concentrations of azamethiphos, deltamethrin and hydrogen peroxide in the present study. This can be explained by the high sensitivity towards azamethiphos and hydrogen peroxide recorded in adult *C. rogercresseyi* in Chile (Agusti and Bravo personal observation, 2014). Azamethiphos was introduced in the salmon industry in

Table 1
Pairwise comparison (*Z* test) of the hatching rate and maturation of unpigmented egg strings (%)between treatments at day seven, exposed to low and high dose of deltamethrin (DTM), emamectin benzoate (EMB), azamethiphos (AZA) and hydrogen peroxide (PER). "s": significant difference ($p < 0.05$); "ns": no significant difference ($p > 0.05$).

Status	Dose	Treatment	%	*Z*	*p*	
Hatching	Low	DTM–PER	67–36	1.88	0.030	s
		DTM–EMB	67–43	1.42	0.078	ns
		DTM–AZA	67–44	1.42	0.077	ns
		AZA–PER	44–36	0.45	0.326	ns
		AZA–EMB	44–43	0.05	0.480	ns
		EMB–PER	43–36	0.39	0.349	ns
	High	DTM–PER	61–50	0.60	0.273	ns
		DTM–EMB	61–42	1.18	0.119	ns
		DTM–AZA	61–50	0.63	0.264	ns
		AZA–EMB	50–42	0.40	0.343	ns
		AZA–PER	50–50	0.00	0.500	ns
		EMB–PER	42.1–50.0	0.43	0.333	ns
Unpigmented	Low	PER–AZA	42.9–31.3	0.66	0.255	ns
		PER–EMB	42.9–21.4	1.25	0.106	ns
		PER–DTM	42.9–14.3	1.87	0.031	s
		AZA–EMB	31.3–21.4	0.62	0.269	ns
		AZA–DTM	31.3–14.3	1.22	0.111	ns
		DTM–EMB	14.3–21.4	0.53	0.296	ns
	High	PER–EMB	50.0–26.3	1.34	0.089	ns
		PER–AZA	50.0–21.4	1.58	0.057	ns
		PER–DTM	50.0–5.6	2.88	0.002	s
		EMB–AZA	26.3–21.4	0.33	0.372	ns
		EMB–DTM	26.3–5.6	1.81	0.035	s
		DTM–AZA	5.6–28.6	1.74	0.041	s

May 2013 and hydrogen peroxide has been only occasionally used. This in contrast to Norway, where the usage has been extensive and resistance has been reported for both drugs (Grøntvedt et al., 2014; Helgesen et al., 2015). Resistance towards deltamethrin has though previously been reported in *C. rogercresseyi* in Chile (Helgesen et al., 2014). In the current study, the high mortality of the gravid females exposed to both deltamethrin concentrations could be explained because they belonged to a sensitive population.

Abortion was also recorded in the hatching egg strings exposed to emamectin benzoate and viable larvae were not found. However, only 10% mortality was recorded in gravid females exposed to 100 ppb emamectin benzoate and 35% mortality at 500 ppb. This is most likely due to a reduced sensitivity of *C. rogercresseyi*, a situation that has been observed since 2006 (Bravo et al., 2008, 2010a). Thus, all treatments had a detrimental effect on the hatching of the exposed eggs strings of sea lice, as well as on the survival of the exposed larvae.

These results are in accordance with personal observations under field conditions in Region X. Unpigmented eggs strings from gravid females collected from a farm during the application of a bath treatment with azamethiphos had a delayed hatching, with low survival of larvae to the copepodid stage (10%). This contrasted with the mature, pigmented egg strings which showed a quick hatching and abortion, without surviving nauplii. A similar situation was reported by Aaen et al. (2014), where a few egg strings with a low level of pigmentation hatched when exposed to a low concentration of hydrogen peroxide (470 ppm) for 30 min. However, none of the nauplii developed into viable copepodides. This in contrast to pigmented egg strings, where none hatched after exposure to the same concentration.

Some of the mechanisms of action of the chemicals used in this study on adult lice are known; however, no information is available about the effect on the larvae or eggs. The organophosphate azamethiphos act through the irreversible inhibition of acetylcholinesterase (AChE) activity (Roth and Richards, 1992; Roth et al., 1996) and results in overstimulation of the nerve impulses leading to spastic paralysis and death of the sea louse (Denholm et al., 2002; Fallang et al., 2004; Jones et al., 1992). The synthetic pyrethroids (deltamethrin and cypermethrin) by altering the normal operation of the voltage-gated sodium channel by slowing its activation and inactivation kinetics, which results in uncontrolled bursts of action potentials leading to nerve exhaustion and death (Fallang et al., 2005; Narahashi, 1992). The avermectin emamectin benzoate works by interfering with nerve transmission; they bind irreversibly to glutamate-gated chloride channels resulting in increased neuron cell membrane permeability to chloride ions at inhibitory synapses, causing paralysis and death (Wolstenholme and Rogers, 2005). The mechanism of action of hydrogen peroxide on sea lice is not well understood but has been speculated to involve a mechanical paralysis resulting from the liberation of oxygen in the gut and hemolymph causing the lice to float up and off the fish (Grant, 2002; Thomassen, 1993). In contrast to the other three products, the hydrogen peroxide does not kill the adult *C. rogercresseyi*; they are only detached from the host, (Bravo et al., 2010b). The same was reported in *L. salmonis* (Johnson et al., 1993). The differences observed on the larval survival, in the hatching abortion or in the level of maturation of the exposed egg strings to the anti-louse products could be explained by these or other mechanisms. In this way, further studies are required to assess the effect of these chemotherapeutic drugs on the reproduction viability of the survival females under field conditions.

Acknowledgments

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References

Aaen, S.M., Aunsmo, A., Horsberg, T.E., 2014. Impact of hydrogen peroxide on hatching ability of egg strings from salmon lice (*Lepeophtheirus salmonis*) in a field treatment and in a laboratory study with ascending concentrations. Aquaculture 422–423, 167–171.
Aaen, S.M., Helgesen, K.O., Bakke, M.J., Kaur, K., Horsberg, T.E., 2015. Drug resistance in sea lice: a threat to salmonid aquaculture. Trends Parasitol. 31 (2), 72–81.
Bravo, S., 2003. Sea lice in Chilean salmon farms. Bull. Eur. Assoc. Fish Pathol. 23, 197–200.
Bravo, S., 2010a. The reproductive output of sea lice *Caligus rogercresseyi* under controlled conditions. Exp. Parasitol. 125, 51–54.
Bravo, S., 2010b. Caligidae ectoparasites on farmed salmonids in Chile. Thesis for the degree of Doctor philosophiae. Norwegian School of Veterinary Science, Oslo 978-82-7725-194-3.
Bravo, S., Sevatdal, S., Horsberg, T., 2008. Sensitivity assessment of *Caligus rogercresseyi* to emamectin benzoate in Chile. Aquaculture 282, 7–12.
Bravo, S., Sevatdal, S., Horsberg, T.E., 2010a. Sensitivity assessment in the progeny of *Caligus rogercresseyi* to emamectin benzoate. Bull. Eur. Assoc. Fish Pathol. 30, 92–98.
Bravo, S., Treasurer, J., Sepulveda, M., Lagos, C., 2010b. Effectiveness of hydrogen peroxide in the control of Caligus rogercresseyi in Chile and implications for sea louse Management. Aquaculture 303, 22–27.
Bron, J.E., Sommerville, C., Wootten, R., Rae, G.H., 1993. Influence of treatment with dichlorvos on the epidemiology of *Lepeophtheirus salmonis* (Krøyer, 1837) and *Caligus elongatus* Nordmann, 1832 on Scottish salmon farms. In: Boxshall, G.A., Defaye, D. (Eds.), Pathogens of Wild and Farmed Fish: Sea Lice. Ellis-Horwood, New York, pp. 263–274.
Denholm, I., Devine, G.J., Horsberg, T.E., Sevatdal, S., Fallang, A., Nolan, D.V., Powell, R., 2002. Analysis and management of resistance to chemotherapeutants in salmon lice *Lepeophtheirus salmonis* (Krøyer) (Copepoda: Caligidae). Pest Manag. Sci. 58, 528–536.
Fallang, A., Ramsay, J.M., Sevatdal, S., Burka, J.F., Jewess, P., Hammell, K.L., Horsberg, T.E., 2004. Evidence for occurrence of an organophosphate-resistant type of acetylcholinesterase in strains of sea lice (*Lepeophtheirus salmonis* Krøyer). Pest Manag. Sci. 60, 1163–1170.
Fallang, A., Denholm, I., Horsberg, T.E., Williamson, M.S., 2005. Novel point mutation in the sodium channel gene of pyrethroid-resistant sea lice *Lepeophtheirus salmonis* (Crustacea: Copepoda). Dis. Aquat. Org. 65, 129–136.
Grant, A.N., 2002. Medicines for sea lice. Pest Manag. Sci. 58, 521–527.
Grøntvedt, R.N., Jansen, P., Horsberg, T.E., Helgesen, K.O., Tarpai, A., 2014. The Surveillance Programme for Resistance to Chemotherapeutants in Salmon Lice (*Lepeophtheirus salmonis*) in Norway 2013. Norwegian Veterinary Institute, Oslo.
Helgesen, K.O., Horsberg, T.E., 2013. Single-dose field bioassay for sensitivity testingin sea lice, *Lepeophtheirus salmonis*: development of a rapid diagnostic tool. J. Fish Dis. 36, 261–272.
Helgesen, K.O., Bravo, S., Sevatdal, S., Mendoza, J., Horsberg, T.E., 2014. Deltamethrin resistance in the sea louse *Caligus rogercresseyi* (Boxshall and Bravo) in Chile: bioassay results and consumption data for anti-parasitic agents with references to Norwegian conditions. J. Fish Dis. 37, 877–890.

Helgesen, K.O., Romstad, H., Aaen, S.M., Horsberg, T.E., 2015. First report of reduced sensitivity towards hydrogen peroxide found in the salmon louse *Lepeophtheirus salmonis* in Norway. Aquac. Rep. http://dx.doi.org/10.1016/j.aqrep.2015.01.001.

Johnson, S.C., Constible, J.M., Richard, L., 1993. Laboratory investigation on the efficacy of hydrogen peroxide against the salmon louse *Lepeophtheirus salmonis* and its toxicological effects on Atlantic salmon *Salmo salar* and Chinook salmon *Oncorhynchus tshwytscha*. Dis. Aquat. Org. 17, 197–204.

Jones, M.W., Sommerville, C., Wootten, R., 1992. Reduced sensitivity of the salmon louse, *Lepeophtheirus salmonis*, to the organophosphate dichlorvos. J. Fish Dis. 15, 197–202.

McAndrew, K.J., Sommerville, C., Wootten, R., Bron, J.E., 1998. The effects of hydrogen peroxide on different life-cycle stages of the salmon louse, *Lepeophtheirus salmonis* (Krøyer, 1837). J. Fish Dis. 21, 221–228.

Narahashi, T., 1992. Overview of toxins and drugs as tools to study excitable membrane ion channels: II. Transmitter activated channels. Methods Enzymol. 207, 643–658.

Pino-Marambio, J., Mordue, A.J., Birkett, M., Carvajal, J., Asencio, G., Mellado, A., Quiroz, A., 2007. Behavioral studies of host, non-host, and mate location by the sea louse, *Caligus rogercresseyi* Boxshall & Bravo, 2000 (Copepoda: Caligidae). Aquaculture 271 (1–4), 70–76.

Reyes, X., Bravo, S., 1983. Nota sobre una copepoidosis en salmones de cultivo. Investig. Mar. Valparaíso 11, 55–57.

Roth, M., Richards, R.H., 1992. Trials on the efficacy of azamethiphos and its safety to salmon for the control of sea lice. In: Michel, C., Alderman, D.J. (Eds.), Chemotherapy in aquaculture: from theory to reality. Office International Des Epizooties, Paris, France, pp. 212–218.

Roth, M., Richards, R.H., Dobson, D.P., Rae, G.H., 1996. Field trials on the efficacy of the organophosphorus compound azamethiphos for the control of sea lice (Copepoda: Caligidae) infestations of farmed Atlantic salmon (Salmo salar). Aquaculture 140, 217–239.

Sevatdal, S., Horsberg, T.E., 2003. Determination of reduced sensitivity in sea lice (*Lepeophtheirus salmonis* Krøyer) against the pyrethroid deltamethrin using bioassays and probit modeling. Aquaculture 218, 21–31.

Thomassen, J.M., 1993. Hydrogen peroxide as a delousing agent for Atlantic salmon. In: Boxshall, G.A., Defaye, D.D. (Eds.), Pathogens of wild and farmed fish: sea lice. Ellis Horwood, New York, pp. 290–295.

Toovey, J.P.G., Lyndon, A.R., 2000. Effects of hydrogen peroxide, dichlorvos and cypermethrin on subsequent fecundity of sea lice, *Lepeophtheirus salmonis*, under fish farm conditions. Bull. Eur. Assoc. Fish Pathol. 20, 224–228.

Treasurer, J.W., Wadsworth, S., Grant, A., 2000. Resistance of sea lice, *Lepeophtheirus salmonis* (Krøyer), to hydrogen peroxide on farmed Atlantic salmon, *Salmo salar* L. Aquac. Res. 31, 855–860.

Wolstenholme, A.J., Rogers, A.T., 2005. Glutamate-gated chloride channels and the mode of action of the avermectin/milbemycin anthelmintics. Parasitology 131, S85–S95.