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(54) Titel: **Treatment of Marine Parasite Infection**

(57) Sammendrag:
combination of an anti-parasitic agent, such as azamethiphos, and fresh water for use in the treatment or prevention of a marine parasite infection in a fish, wherein the use is sequential, separate and/or simultaneous. Typically, the use is sequential, and the antiparasitic agent is applied after an initial period in the fresh water. The treatment is suitable for a variety of fish, for example Salmo salar, sea bass, sea bream, and is effective against parasitic infections such as those mediated by Lepeophtheirus salmonis, Caligus rogercresseyi, Caligus clemensi and amoebic gill disease. The combination also provides methods of enhancing the effectiveness of an anti-marine parasitic agent, reducing the resistance of marine parasites to an anti-marine parasitic agent, treating a marine parasite infection in a fish, and preventing re-infection of a fish with a marine parasite.

Treatment of Marine Parasite Infection

The present invention relates to a combination of an anti-parasitic agent and fresh water for use in the treatment or prevention of a marine parasite infection in a fish. The invention also provides methods of enhancing the effectiveness of an anti-marine parasitic agent, reducing the resistance of marine parasites to an anti-marine parasitic agent, treating a marine parasite infection in a fish, and preventing re-infection of a fish with a marine parasite.

Marine ectoparasite infestation in farmed fish, particularly salmon, has posed a constant challenge to commercial fish farming for many decades. Marine parasites include *Neoparamoeba perurans*, gill flukes, skin flukes and Cymothoidae. Parasitic sea lice arguably present the most significant challenge at present to control and treat in Atlantic salmon.

The term sea lice refers to a number of closely related species of parasitic copepods in the family *Caligidae* (caligid copepods). There are three major genera of sea lice: *Pseudocaligus*, *Caligus* and *Lepeophtheirus*. The species *Lepeophtheirus salmonis* is responsible for most disease outbreaks on farmed salmonids and is responsible for indirect and direct losses in aquaculture in excess of US \$100 million annually. In the southern hemisphere, the species *Caligus rogercresseyi* is more prevalent.

The life cycle of Caligid copepods consists of two free-living planktonic *nauplius* stages, one free-swimming infectious *copepodid* stage, four to six attached *chalimus* stages, one or two pre-adult stages, and one adult stage. Each of these developmental stages is separated by a moult. Once the adult stage is reached, caligid copepods do not undergo additional moults. In the case of *L. salmonis*, eggs hatch into the free-swimming first nauplius stage, which is followed by a second nauplius stage, and then the infectious copepodid stage. Once the copepodid locates a suitable host fish it continues its development through four chalimus stages, first and second pre-adult stages, and then a final adult stage.

Several management strategies are currently used to reduce the intensity of marine parasite infestations. These include fallowing of sites prior to restocking, year class separation and

selection of farm sites to avoid areas where there are high densities of wild hosts or other environmental conditions suitable for parasitic establishment. Although the use of these strategies can in some cases lessen parasitic infection rates, their use individually or in combination has not been effective in eliminating infection.

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A variety of chemical treatments have also been used to control parasites in fish farms. These include compounds such as hydrogen peroxide, organophosphates (e.g., dichlorvos and azamethiphos), ivermectin (and related compounds such as emamectin benzoate), insect moulting regulators and pyrethrins. Most marine parasite treatments can be classified into groups including treatments administered by bath (e.g. organophosphates, pyrethrins) and treatments administered orally (e.g. ivermectin).

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Particularly for the treatment of sea lice, chemicals used in bath treatments are not necessarily effective against all of the stages of sea lice found on fish.

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For example, azamethiphos has been found to effectively kill adult and pre-adult sea lice (mobile stage), but has been found to be relatively ineffective at killing juvenile sea lice (attached stage). Azamethiphos is an organophosphate that acts as an acetylcholinesterase inhibitor. Juvenile stage sea lice are thought to have no or low levels of cholinesterase, and therefore are not significantly affected by this treatment.

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Furthermore, there is increasing evidence for the development of resistance in marine parasites such as sea lice to some of these treatments, especially in frequently-treated populations.

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Fresh water treatments have been found to kill all stages of sea lice. However, the required treatment times are in the range of 7-9 hours. Thus, for example, Stone *et al.* (Journal of Fish Diseases 25: 371-373, 2002) indicates that exposure to fresh water for three hours is an ineffective treatment for controlling sea lice in fish. Furthermore, often not all sea lice are removed or killed after fresh water treatment. Due to the length of treatment time required, fresh water treatment has generally been accepted in the industry as an inefficient de-lousing treatment, with chemical baths and immunogenic treatments being preferred.

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There therefore remains a need for improved or alternative treatments for marine parasite infections in fish, particularly for parasites that have become resistant to existing treatments.

- 5 Accordingly, a first aspect of the invention provides a combination of an anti-parasitic agent and fresh water for use in the treatment or prevention of a marine parasite infection in a fish, wherein the use is sequential, separate and/or simultaneous.

10 In an embodiment of the invention, the use is sequential, and the anti-parasitic agent is applied after an initial period in the fresh water. The anti-marine parasitic agent may be applied in fresh water, brackish water or sea water, preferably fresh water.

The present invention has been found to enhance the effectiveness of anti-parasitic agents against all stages of sea lice (attached, mobile, juvenile, pre-adult & adult lice). The present
15 invention provides a total treatment time of less than half of known fresh water treatments and with an efficacy of 100% removal of sea lice, even of sea lice that are genetically resistant to known chemical treatments.

The present invention has been found to prevent resettlement of sea lice and thus prevent
20 reinfection of fish treated according to the method. The effectiveness of the present invention will help to prevent resistance development to chemical bath treatments.

In embodiments of the present invention, the fresh water has a salinity of less than 29 ppt, less than 20 ppt, less than 10 ppt, less than 5 ppt, or less than 2 ppt. In particular
25 embodiments, the fresh water has a salinity of around 0 ppt, or 0 ppt (i.e. no measurable salinity).

The initial period in the fresh water may be at least 15 minutes, at least 30 minutes, at least 1 hour, at least 2 hours, or at least 3 hours. The initial period in the fresh water may be less
30 than 4 hours, less than 5 hours, less than 6 hours, or less than 7 hours. The initial period may be longer than 6 or 7 hours, but this would typically achieve no further technical effect.

The anti-parasitic agent should typically be applied for the period of time recommended for the agent as a standalone treatment agent (i.e. for a treatment regime without an initial period in fresh water; the label indicated dose time). In practice, the anti-parasitic agent
 5 may be applied for at least 15 minutes, at least 30 minutes, at least 1 hour, at least 2 hours, or at least 3 hours. The anti-parasitic agent may be applied for less than 4 hours, less than 5 hours, less than 6 hours, or less than 7 hours.

In a particular embodiment of the invention, the fish are held in fresh water for an initial
 10 period of around 2 hours, then the anti-parasitic agent is applied in fresh water for around 1 hour.

In embodiments of the invention, the fish is a salmonid, preferably *Salmo salar*. In other embodiments, the fish is sea bass, sea bream, *Seriola* sp., grouper, Asian sea bass, cod,
 15 lump sucker fish (*Cyclopteridae*), or Wrasse sp.

The anti-parasitic agent may be one or more agents selected from the group consisting of: an organophosphate; azamethiphos; diclorvos; pyrethroid; deltamethrin; cypermethrin; pyrethrin; carbamate; eonicitinoid; imidacloprid; thiocloprid; avermectins; emamectin;
 20 doramectin; ivermectin; spinosad; teflubenzuron; di-flubenzuron; lufenuron; hexaflumuron; milbamectin; cyromazine; praziquantel; formalin; hydrogen peroxide; boric acid; propionic acid; and bronopol. In a preferred embodiment, the anti-parasitic agent is azamethiphos.

The anti-parasitic agent is applied at 5% to 500% of the recommended treatment dose. For
 25 example, in an embodiment of the invention, the anti-parasitic agent is azamethiphos, which is applied at a dose of 0.005 to 0.5 mg/L or 0.05 to 0.2 mg/L, for example around 0.1 mg/L, which is the recommended treatment dose for sea lice (the label indicated dose is 0.2 mg/L for Salmosan[®] Vet, which is 50 % w/w azamethiphos).

30 In embodiments of the invention, the parasite is one or more parasites selected from the group consisting of: a copepod; *Lepeophtheirus* sp.; *Caligus* sp.; *Lepeophtheirus salmonis*; *Caligus rogercresseyi*; *Caligus clemensi*; amoebic gill disease (*Neoparamoeba perurans*);

gill fluke (monogeneans); *Dactylogyrus* sp.; skin fluke; *Gyrodactylus* sp.; *Cymothoidae*; and *Cymothoa exigua*. In preferred embodiments the parasite is *Lepeophtheirus salmonis*, *Caligus rogercresseyi* or *Caligus clemensi*.

- 5 A second aspect of the invention provides a method of enhancing the effectiveness of an anti-marine parasitic agent, the method comprising the steps: (i) contacting the marine parasite with fresh water; and (ii) contacting the marine parasite with the anti-marine parasitic agent. Optionally, step (ii) follows step (i).

- 10 Thus, the invention provides a more reliable effective treatment.

- A third aspect of the invention provides a method of reducing the resistance of marine parasites to an anti-marine parasitic agent, the method comprising the steps: (i) contacting the marine parasite with fresh water; and (ii) contacting the marine parasite with the anti-
15 marine parasitic agent. Optionally, step (ii) follows step (i).

Thus, the invention provides an effective treatment for parasites that have developed a genetic or other resistance to an anti-marine parasitic agent.

- 20 A fourth aspect of the invention provides a method of treating a marine parasite infection in a fish, the method comprising the steps: (i) contacting the infected fish with fresh water; and (ii) contacting the infected fish with an anti-marine parasitic agent. Optionally, step (ii) follows step (i).

- 25 A fifth aspect of the invention provides a method of preventing re-infection of a fish with a marine parasite, the method comprising the steps: (i) contacting the infected animal with fresh water; and (ii) contacting the infected animal with an anti-marine parasitic agent. Optionally, step (ii) follows step (i).

- 30 The optional features of the invention described in respect of the first aspect of the invention are optional features for the second, third, fourth and fifth aspects of the invention as appropriate.

Examples**Example 1**

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Tests were carried out on a well-boat comprising two 550 m³ wells; the first well at starboard side (SB) and the second well at port side (PS).

Atlantic salmon averaging 600-700 g were loaded from the sea into SB and PS wells containing fresh water having an initial salinity of 0 ppt (grams of salt in 1 kilogram of seawater).

32,401 fish were loaded into the PS well over a period of 50 minutes. 33,101 fish were loaded into the SB well over a period of 44 minutes. After loading, the fish were treated for 2 hours in the fresh water. Salmosan[®] Vet (azamethiphos 50% w/w powder) was then added to the fresh water at a concentration of 0.2 mg/L and the fish were treated for a further 60 minutes. No visible stress was observed in the fish post-treatment.

CO₂ strippers and 4 kg of bicarbonate of soda were used to control the pH in the well water to levels appropriate for the well-being of the fish. The pH was maintained to around 6.35. After the treatment, the fish were released from the PS and SB wells to a new saltwater site.

Table 1 shows the average number of sea lice on each fish pre- and post-treatment at the stated four stages of the lice lifecycle. For example, a count of 0.2 indicates an average of one attached sea louse per five fish.

Table 1

	L. Fem Gravid	L. Ad. Fem.	L. Male	L. Pre-adult
Pre-treatment	0.2	0.7	1.05	0.75
Post-treatment	0	0.1	0	0.1

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The treatment showed 100% clearance of sea lice infection up to 2 days post-treatment with no resettlement on the fish. A few sea lice were still attached post-treatment, but these were picked off and no recovery of the sea lice was observed.

- 5 A control comprising 7 cages stocked with Atlantic salmon receiving Salmosan[®] Vet (azamethiphos 50% w/w powder) and H₂O₂ was also trialled in parallel, but in contrast only achieved an average of 70% reduction in sea lice infection.

10 Very low mortality rates were observed in the fish population post-treatment. The fish appeared to show less signs of stress when compared to fish treated with Salmosan[®] Vet in seawater for an hour.

Example 2

- 15 Tests were carried out on a well-boat that was different to that used for Example 1, which boat comprised two 550 m³ wells; the first well at starboard side (SB) and the second well at port side (PS).

20 Atlantic salmon were stocked into both the SB and PS wells. 35,343 fish averaging 1.45 kg were loaded into the PS well containing water having an initial salinity of around 2 ppt. 19,922 fish averaging 836 g were loaded into the SB well containing an initial salinity of around 4 ppt.

- 25 After loading, the fish were treated for about 2 hours in the fresh water wells. Salmosan[®] Vet (azamethiphos 50% w/w powder) was then added to the fresh water at a concentration of 0.2 mg/L and the fish were left for about 60 minutes further. No visible stress was observed in the fish post-treatment.

30 The pH, salinity and CO₂ levels in the well water were monitored and maintained throughout the treatment.

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As no CO₂ stripper was available on the well-boat, a total of 75 kg of bicarbonate of soda was used to regulate the pH. The resulting pH was between 6.5 and 7.5. The salinity remained at 4 ppt for the starboard well, and varied only slightly (within 1 ppt unit) for the port side well. Oxygen levels were also monitored and adjusted to the appropriate level as required. The CO₂ level was measured as 40.98 mg/L for the port side well, and as 36.79 mg/L for the starboard well.

100% clearance of sea lice from the fish was observed post-treatment with low mortality rates in the fish population. After the treatment, the fish were released from the PS and SB wells at a new saltwater site.

Example 3

Live sea lice were collected from a Scottish marine location, and were transported and held overnight for at least 18 hours at 10°C in gently aerated normal seawater to ensure that only those lice that were considered healthy and active were studied ($\approx$ 80% male to 20% female).

Each of two perforated holding chambers were stocked with lice, and were immersed in 1-litre beakers containing test solutions of varying salinities 0 ppt (freshwater), 9 ppt, 18 ppt and 35 ppt (normal seawater) for varying periods of time, from 30 to 240 minutes (table 2) prior to treatment with nil active (control), azamethiphos (0.1 ppm) or imidacloprid (20 ppm). After treatment for either 30 or 60 minutes, lice were rinsed and subsequently placed in beakers containing fresh, filtered, aerated normal seawater.

After treatment, lice were maintained in normal, aerated seawater at 10°C and assessed as live, moribund or dead after 24 hours. Sea lice were assessed as live according to following criteria:

- exhibit normal swimming behaviour;
- swim off when picked up on forceps;
- strong adhesion to Petri dish;
- adhere or quickly re attach to dish after swirling for 10 seconds; and

- tail held horizontally.

Sea lice were assessed as moribund according to following criteria:

- 5
 - abnormal swimming behaviour: feeble movements, spiralling motion or prolonged floating;
 - swimming response absent or limited when picked up on forceps;
 - unable to maintain strong adhesion to Petri dish;
 - failure to adhere or quickly re attach to dish after swirling for 10 seconds; and
- 10
 - tail curved.

Sea lice were assessed as dead if they exhibited an absence of any movement.

The results of the study are presented in Tables 2 and 3.

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Table 2

Salinity	Treatment time	Active compound	Total lice	Observed N				Observed %			
				Live	Moribund	Dead	Affected	Live	Moribund	Dead	Affected
35 ppt		Nil (Control 1)	22	19	3	0	3	86%	14%	0%	14%
35 ppt		Nil (Control 2)	22	18	4	0	4	82%	18%	0%	18%
35 ppt	30 mins	Aza (0.1 ppm)	22	14	8	0	8	64%	36%	0%	36%
35 ppt	60 mins	Aza (0.1 ppm)	22	13	9	0	9	59%	41%	0%	41%
35 ppt	60 mins	Imid (20 ppm)	22	0	22	0	22	0%	100%	0%	100%
18 ppt	60 mins	Nil (18 ppt)	22	13	8	1	9	59%	36%	5%	41%
18 ppt	60 mins	Aza (0.1 ppm)	22	9	12	1	13	41%	55%	5%	59%
18 ppt	60 mins	Imid (20 ppm)	22	0	22	0	22	0%	100%	0%	100%
18 ppt	120 mins	Nil (18 ppt)	22	11	11	0	11	50%	50%	0%	50%
18 ppt	120 mins	Aza (0.1 ppm)	22	11	11	0	11	50%	50%	0%	50%
18 ppt	120 mins	Imid (20 ppm)	22	0	22	0	22	0%	100%	0%	100%
18 ppt	240 mins	Nil (18 ppt)	22	9	13	0	13	41%	59%	0%	59%
18 ppt	240 mins	Aza (0.1 ppm)	22	8	14	0	14	36%	64%	0%	64%
18 ppt	240 mins	Imid (20 ppm)	22	0	22	0	22	0%	100%	0%	100%
9 ppt	30 mins	Nil (9 ppt)	22	15	6	1	7	68%	27%	5%	32%
9 ppt	30 mins	Aza (0.1 ppm)	22	11	11	0	11	50%	50%	0%	50%
9 ppt	60 mins	Nil (9 ppt)	22	12	7	3	10	55%	32%	14%	45%
9 ppt	60 mins	Aza (0.1 ppm)	22	13	6	3	9	59%	27%	14%	41%

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9 ppt	120 mins	Nil (9 ppt)	22	13	9	0	9	59%	41%	0%	41%
9 ppt	120 mins	Aza (0.1 ppm)	22	8	11	3	14	36%	50%	14%	64%
9 ppt	180 mins	Nil (9 ppt)	22	9	11	2	13	41%	50%	9%	59%
9 ppt	180 mins	Aza (0.1 ppm)	22	4	14	4	18	18%	64%	18%	82%
9 ppt	240 mins	Nil (9 ppt)	23	5	13	5	18	22%	57%	22%	78%
9 ppt	240 mins	Aza (0.1 ppm)	22	0	8	14	22	0%	36%	64%	100%
0 ppt	30 mins	Nil (0 ppt)	22	12	10	0	10	55%	45%	0%	45%
0 ppt	30 mins	Aza (0.1 ppm)	22	16	5	1	6	73%	23%	5%	27%
0 ppt	60 mins	Nil (0 ppt)	22	11	11	0	11	50%	50%	0%	50%
0 ppt	60 mins	Aza (0.1 ppm)	22	13	9	0	9	59%	41%	0%	41%
0 ppt	60 mins	Imid (20 ppm)	22	0	21	1	22	0%	95%	5%	100%
0 ppt	120 mins	Nil (0 ppt)	22	0	18	4	22	0%	82%	18%	100%
0 ppt	120 mins	Aza (0.1 ppm)	22	0	20	2	22	0%	91%	9%	100%
0 ppt	120 mins	Imid (20 ppm)	22	0	21	1	22	0%	95%	5%	100%
0 ppt	240 mins	Nil (0 ppt)	32	0	25	7	32	0%	78%	22%	100%
0 ppt	240 mins	Aza (0.1 ppm)	23	0	12	11	23	0%	52%	48%	100%
0 ppt	240 mins	Imid (20 ppm)	22	0	13	9	22	0%	59%	41%	100%

Nil (Control 1) and Nil (Control 2) are sea water (35 ppt) controls; live = unaffected; affected = moribund plus dead; Aza = azamethiphos; Imid = imidocloprid.

- 5 There were statistically significant differences in numbers of 'affected' lice for all treatment regimens other than 0.1 ppm azamethiphos for 30 min at both 35 ppt and 0 ppt. The result for 9 ppt for 30 min was also not significantly different from the controls. The following low salinity conditions without active are efficacious: 18 ppt (for 60, 120 and 240 min), 9 ppt (for 30, 60, 120, 180 and 240 min) and 0 ppt (30 and 60 min). Exposure to 0 ppt for 120 or
- 10 240 min is as efficacious as 20 ppm imidacloprid.

When compared to the appropriate salinity control, adding 0.1 ppm azamethiphos had a significant additional effect when lice were pre-exposed to 9 ppt for 240 min. This effect was not seen at the lower test salinity of 0 ppt due to the overall efficacy of this low salinity

15 on its own.

Due to the strong adverse effect of adding imidacloprid at 20 ppm (resulting in 100% efficacy at all tested salinities), it was impossible to comment further on any potential interactions with pre-exposure to low salinity.

Example 4

Live sea lice were collected from a Scottish marine location, and were transported and held overnight for at least 18 hours at 10°C in gently aerated normal seawater to ensure that only those lice that were considered healthy and active were studied ($\approx$ 80% male / 20% female).

Each of two or three perforated holding chambers were stocked with lice, and were immersed in 1-litre beakers containing test solutions of sea-water at 35 ppt containing azamethiphos at 0.1 ppm or 0.3 ppm. After treatment for 60 minutes, lice were rinsed and placed in beakers containing fresh seawater for a further 180 minutes. Lice were then rinsed and subsequently placed in beakers containing fresh seawater.

After treatment, lice were maintained in normal, aerated seawater at 10°C and assessed as live, moribund or dead after 24 hours according to the criteria of Example 3.

The results of the study are presented in Table 3.

Table 3

Salinity	Active compound	Total lice	Observed N				Observed %			
			Live	Moribund	Dead	Affected	Live	Moribund	Dead	Affected
35 ppt	Aza (0.1 ppm)	30	13	17	0	17	43%	57%	0%	57%
35 ppt	Aza (0.3 ppm)	29	1	28	0	28	3%	97%	0%	97%
9 ppt	Aza (0.1 ppm)	31	3	26	2	28	10%	84%	6%	90%
9 ppt	Aza (0.3 ppm)	41	0	21	20	41	0%	51%	49%	100%
4.5 ppt	Aza (0.1 ppm)	29	0	26	3	29	0%	90%	10%	100%
4.5 ppt	Aza (0.3 ppm)	37	0	6	31	37	0%	16%	84%	100%
0 ppt	Aza (0.1 ppm)	33	0	17	16	33	0%	52%	48%	100%
0 ppt	Aza (0.3 ppm)	31	0	0	31	31	0%	0%	100%	100%

Live = unaffected; affected = moribund plus dead; Aza = azamethiphos; Imid = imidocloprid.

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When considering live versus affected lice there are no statistically significant differences apparent within the data set within any salinity regime except for 0.1 ppm azamethiphos versus 0.3 ppm at 35 ppt where numbers of affected lice are significantly less ($p < 0.001$).

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Amongst other things, these data show that exposure to low salinity after treatment with 0.1 ppm azamethiphos was as efficacious at affecting sea lice as treatment with 0.3 ppm azamethiphos at a salinity of 35 ppt. Additionally, it is apparent that varying salinity has little or no additive effect on affecting sea lice when high doses of azamethiphos are utilised.

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Claims

1. A combination of an anti-parasitic agent and fresh water for use in the treatment or prevention of a marine parasite infection in a fish, wherein the use is sequential, separate
5 and/or simultaneous.
2. The combination for use according to claim 1, wherein the use is sequential, and the anti-parasitic agent is applied after an initial period in the fresh water.
- 10 3. The combination for use according to claim 1 or claim 2, wherein the anti-parasitic agent is applied in fresh water, brackish water or sea water.
4. The combination for use according to any preceding claim, wherein the fresh water has a salinity of less than 29 ppt, less than 5 ppt, or less than 2 ppt.
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5. The combination for use according to any preceding claim, wherein the fresh water has a salinity of around 0 ppt, or 0 ppt.
6. The combination for use according to any one of claims 2 to 5, wherein the initial
20 period in the fresh water is at least 30 minutes, at least 1 hour, at least 2 hours, or at least 3 hours.
7. The combination for use according to claim 6, wherein the initial period in the fresh water is less than 4 hours, or less than 5 hours
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8. The combination for use according to any one of claims 2 to 5, wherein the anti-parasitic agent is applied for at least 30 minutes, at least 1 hour, at least 2 hours, or at least 3 hours.
- 30 9. The combination for use according to claim 6, wherein the anti-parasitic agent is applied for less than 4 hours, or less than 5 hours.

10. The combination for use according to any one preceding claim, wherein the fish are held in fresh water for an initial period of around 2 hours, then the anti-parasitic agent is applied in fresh water for around 1 hour.
- 5 11. The combination for use according to any one preceding claim, wherein the fish is a salmonid, preferably *Salmo salar*.
12. The combination for use according to any one of claims 1 to 10, wherein the fish is sea bass, sea bream, *Seriola* sp., grouper, Asian sea bass, cod, lump sucker fish
10 (*Cyclopteridae*), or Wrasse sp.
13. The combination for use according to any one preceding claim, wherein the anti-parasitic agent is one or more agents selected from the group consisting of: an organophosphate; azamethiphos; diclorvos; pyrethroid; deltamethrin; cypermethrin;
15 pyrethrin; carbamate; eonicitinoid; imidacloprid; thiocloprid; avermectins; emamectin; doramectin; ivermectin; spinosad; teflubenzuron; di-flubenzuron; lufenuron; hexaflumuron; milbamectin; cyromazine; praziquantel; formalin; hydrogen peroxide; boric acid; propionic acid; and bronopol.
- 20 14. The combination for use according to any one preceding claim, wherein the anti-parasitic agent is applied at 5% to 500% of the recommended treatment dose.
15. The combination for use according to any one preceding claim, wherein the anti-parasitic agent is azamethiphos applied at a dose of 0.005 to 0.5 mg/L.
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16. The combination for use according to any one preceding claim, wherein the parasite is one or more parasites selected from the group consisting of: a copepod; *Lepeophtheirus* sp.; *Caligus* sp.; *Lepeophtheirus salmonis*; *Caligus rogercresseyi*; *Caligus clemensi*; amoebic gill disease (*Neoparamoeba perurans*); gill fluke (monogeneans);
30 *Dactylogyrus* sp.; skin fluke; *Gyrodactylus* sp.; *Cymothoidae*; and *Cymothoa exigua*.