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(54) **Title**: FISH LICE TREATMENT METHOD

(57) **Abstract**: Fish lice treatment method The present invention provides methods for treating ectoparasitic infections in fish, especially fish lice infections in farmed salmon. Particulate lime is contacted with water housing the ectoparasites and, optionally, their host fish in an amount and for a time sufficient to detach and/or kill the ectoparasites.

# Fish lice treatment method

This invention relates to methods for the treatment of fish, especially farmed salmonid fish, to combat infection thereof by ectoparasites such as fish lice. The invention further relates to methods of killing fish ectoparasites and to compositions for use in the methods of the invention.

Ectoparasites are a widespread and serious problem in aquaculture. In the production of farmed fish, ectoparasites such as fish lice can cause widespread damage to fish, significantly reduced productivity and in severe cases complete collapse of fish stocks. For example, it has been estimated that the annual cost of sea lice to the salmonid farming industry worldwide is around 0.1-0.2 Euro per kg of fish produced. Globally, these costs run into hundreds of millions of US dollars and reflect a significant proportion, around 6%, of the entire production value for salmonids (Costello, *J. Fish Diseases*, 2009, **32**, p.115-118).

Fish ectoparasites typically cause damage at the site of attachment due to physical feeding and enzymatic injection and infected fish generally have increased stress response, reduced immune function and reduced growth rates. There is also significant concern that high concentrations of infected fish in fish farms result in a reservoir of ectoparasites which can infect and seriously damage local populations of wild fish.

Numerous control measures and chemical interventions are used to try and minimise the impact of ectoparasites on fish populations. Many countries recommend or require monitoring programs to identify dangerous levels of parasite infections and a number of chemical agents are known for use against infections. However, good husbandry techniques alone are generally insufficient to keep parasite levels below those that cause health or environmental concerns and so the use of parasiticides to control infections is widespread. Unfortunately, such parasiticides are often highly toxic to the aquatic environment and must be rigorously controlled to prevent their escape. Conventional parasiticides can also suffer from resistance problems. Furthermore, these compounds are often toxic to humans and other animals that eat the fish and this limits the use of parasiticides shortly before harvesting of farmed fish. The cost of conventional parasiticides is also considerable and may be ranked as one of the key factors affecting the profitability of fish farming operations.

Anti-parasitic agents are typically administered in one of two ways - infected fish are either bathed in a solution of the drug (this is typically a short-term treatment in which infected fish are contacted with the drug for a short period of time, e.g. seconds or minutes, before being transferred to clean water) or are fed the drug in their food. Bath treatments administer a known concentration of paraciticide to infected fish for a specified period of time; however such treatments are time- and labour-intensive and can stress the fish. It is also difficult to treat a large area of farm quickly to prevent re-infection. The use of chemical treatments may also pose an environmental risk to the local marine ecosystem. In-feed treatments, such as avermectins, are simpler and less time-intensive to administer and generally pose less of an environmental risk. However, it can be difficult to tailor the drug dose because of differences in feed uptake by the fish. Orally-administrable drugs are also typically expensive to buy and formulate into fish feeds.

A number of simple chemical agents are known as topical disinfectants in aquaculture. Hydrogen peroxide may be used as a short-term bath for removing mobile sea lice from fish. However, care must be taken to control the administration parameters (time, temperature, concentration, etc.) because the agent is toxic to fish. Lime milk, finely divided calcium hydroxide, $Ca(OH)_2$, may be used as a very short-term immersion bath (around 20 seconds) to detach leeches from fish. However, lime milk is toxic to fish and is especially unsuitable for use with fish having sensitive gills, e.g. pike and trout.

There thus exists an acute need in the fish farming industry for an anti-ectoparasitic agent which is cheap, easy to administer and non-toxic to the aquatic environment. The present invention addresses these needs.

The present inventors have surprisingly discovered that particulate lime, when added to water housing fish infected with ectoparasites, can effectively remove or kill the ectoparasites without significantly affecting the health of the fish. This finding provides the basis for an effective anti-parasitic treatment for fish which is simple and cheap to administer as well as having a minimal overall environmental impact.

Thus, in a first aspect, the invention provides a method of treating an ectoparasitic infection in fish, the method comprising contacting water housing said fish with particulate lime, e.g. in a sufficient quantity to detach and/or kill the ectoparasites.

In a related aspect, the invention provides a method of treating the aqueous environment of a fish farm to kill fish ectoparasites in said environment, the method comprising contacting water of the aqueous environment with particulate lime, wherein said water houses farmed fish.

In a further related aspect, the invention provides a method of killing fish ectoparasites comprising contacting water housing said ectoparasites with particulate lime, e.g. water further housing farmed fish which are a host for said ectoparasites.

By the term "housing", "houses" and the like is meant that the aqueous environment (e.g. fresh water or seawater) harbours the fish and/or their ectoparasites at the time of treatment with the particulate lime. It should be understood that the methods of the invention relate to treating the immediate aqueous environment of the fish and/or ectoparasites, e.g. the treatments of the invention not only contact the aqueous environment with said particulate lime but preferably also contact the said fish and/or ectoparasites with said particulate lime, for example by virtue of the particles settling in the water onto the fish and/or their ectoparasites.

The methods described herein relate especially to killing fish ectoparasites and/or detaching ectoparasites from host fish. Ectoparasites may be targeted in their mature or larval stages, but are preferably targeted once they have attached to their hosts because the present methods provide means for killing and/or detaching the ectoparasites without harming the host fish.

Ectoparasites which may be targeted according to the present invention include copepod parasites, especially of the family *Caligidae*. The invention may particularly be applied in relation to sea lice, e.g. of the genera *Lepeophtheirus* and/or *Caligus*, especially *Lepeophtheirus salmonis*, *Caligus rogercresseyi*, *Caligus chiastos*, *Caligus clemensi*, *Caligus epidemicus* and/or *Caligus elongates*. Other ectoparasites which may be targeted include branchurian fish lice of the order *Arguloida*, e.g. Argulus species such as *Arguloida foliaceus* and *Arguloida coregoni*.

Particularly important examples of ectoparasites for treatment are those which infect farmed fish, especially farmed salmon, e.g. *L. salmonis* and *C. rogercresseyi*. In one embodiment, the parasite to be targeted is not a leech, e.g. *Piscicola geometra*.

In one embodiment, the ectoparasites targeted include more than one type (e.g. species) of ectoparasite, for example two, three, or four types of ectoparasite, such as those listed above.

Fish to be treated according to the present invention include wild and farmed fish, but the invention is particularly applied to captive fish, e.g. farmed fish maintained in conventional enclosures such as tanks, cages, nets or the like. This allows for an accurate treatment by addition of a measured amount of particulate lime into the water housing the fish, e.g. onto the surface of the water. The species of fish to be treated will typically be one that is susceptible to infection by an ectoparasite as defined herein, especially a fish species which does not mount an immune response to reject attachment of the ectoparasite. The method of the invention may also be used to treat fish with sensitive gills such as pike and trout. Both seawater and freshwater fish may be treated. Fish that naturally migrate between seawater and fresh water may also be treated in either environment.

Fish that can be treated according to the invention include the salmonids, e.g. Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*), brown trout and sea trout (*Salmo trutta*), arctic char (*Salvelinus alpinus*), and all species of Pacific salmon (e.g. *Oncorhynchus kisutch*, *Oncorhynchus keta* and *Oncorhynchus gorbuscha*). Other fish include carp (family *Cyprinidae*), especially Common carp (*Cyprinus carpio*), Asian carp (e.g. *Hypophthalmichthys nobi*) and Indian carp (e.g. *Gibelion catla*, *Labeo rohita* and *Cirrhinus cirrhosus*) and pike, e.g. Northern pike (*Esox lucius*), as well as bluefin tuna (*Thunnus maccoyi*). The invention is particularly suited to the treatment of farmed Atlantic salmon, especially to the treatment of sea lice (e.g. *L. salmonis* and/or *C. rogercresseyi*) in said salmon.

In one embodiment, the fish to be treated includes more than one type (e.g. species) of fish, for example two, three, or four types of fish of the types listed above.

The term "lime" according to the present invention denotes a calcium-containing inorganic material which comprises (e.g. consists essentially of) calcium oxide (CaO) and/or calcium hydroxide ($Ca(OH)_2$) and which may comprise other components including carbonates, oxides and hydroxides of calcium, as well as silicon, magnesium, aluminium and iron compounds. Preferably, the lime comprises a major portion of CaO, especially at least about 50%, at least about 70%, 80% or 90% by weight CaO, especially greater than 95% by weight CaO. Alternatively, the lime comprises a major portion of $Ca(OH)_2$, especially at least about 50%, at least about 70%, 80% or 90% by weight $Ca(OH)_2$. In an especially preferred embodiment the lime is predominantly CaO, e.g. about 92%, about 95% or about 96% by weight CaO, or the lime is CaO, e.g. quicklime or burnt lime. In a preferred embodiment, the lime for use according to the invention comprises only a minor portion, e.g. less than 50%, less than 25%, less than 5%, less than 2% or less than 0.5% by weight of $Ca(OH)_2$. In one embodiment, the lime is essentially free from $Ca(OH)_2$.

In one embodiment of the invention , the lime is largely free (e.g. substantially free) from impurities. In one embodiment, the residual amount of $CO_2$ in the lime is less than 10%, preferable less than 5%, 4% or 3% by weight, e.g. about 2% by weight. In another embodiment the lime has less than 10% by weight of inorganic oxides other than calcium oxide, especially less than 5%, 4% or 3% of such other inorganic oxides. These other inorganic oxides may be selected from one, more than one, or all of: MgO (preferably less than 1% by weight); $SiO_2$ (preferably less than 1% by weight); $Al_2O_3$ (preferably less than 2% by weight); $Fe_2O_3$ (preferably less than 0.2% by weight); MnO (preferably less than 0.01% by weight); $K_2O$ (preferably less than 0.1% by weight); and $SO_3$ (preferably less than 0.5% by weight). The lime may also be substantially free from inorganic elements other than calcium, especially metallic and metalloid elements which may be toxic if released into the aquatic environment. The lime of the invention preferably comprises less than 20 ppm, especially less than 10 ppm or 5 ppm of these elements. In an especially preferred embodiment, the lime comprises less than 2 ppm arsenic, less than 4 ppm lead, less than 0.2 ppm cadmium and/or less than 0.02 ppm cadmium. Methods for determining the composition of lime are known in the art, including wet chemical methods for determining the CaO and $CO_2$ levels and spectroscopic methods for elemental analysis (e.g. atomic absorption spectrometry, AAS, and inductively coupled plasma atomic emission spectroscopy, ICP-AES).

Lime for use according to the invention may be obtained from commercial sources or may be produced directly. For example, calcium oxide may be produced by thermal decomposition of materials containing calcium carbonate ($CaCO_3$), such as limestone, using methods well established in the art. A preferred source of lime for use according to the invention is from Franzefoss Miljøkalk AS (Norway). Especially preferred for use according to the present invention is lime having the characteristics set out in Example 8 (especially in Table 6). Calcium hydroxide may be produced by contacting (or "slaking") calcium oxide-containing materials with water. Lime obtained from commercial sources will typically require treatment including heating and/or slaking, and particle size alteration (e.g. by sieving or grinding) to provide particulate lime particularly suitable for use according to the invention.

The lime used according to the present invention is particulate lime. The term "particulate" is used to mean that the lime is in a divided solid state, for example as a granulate or powder, or is in the form of a suspension of the solid material, e.g. in an aqueous or non-aqueous liquid such as water, brine or oil. The measurement of particle sizes and the preparation of fractions with given particle sizes may be performed using techniques known in the art. For example, particle sizes may be determined by sieving, optical analysis, electroresistance techniques, laser diffraction and the like. Preparation of particles with given sizes may, for example, be prepared by crushing, milling, grinding and the like, optionally followed by filtration, sedimentation, centrifugation etc. One particularly suitable process for the production of particulate lime for use in the present invention is air classification. In this process, particulate material (e.g. crushed, screened, sized and/or milled material) having a range of particle sizes is separated into fractions having a well-defined range of sizes using a cyclonic air stream. Air classifiers suitable for preparing particulate lime fractions having sizes defined herein are commercially available. The use of cyclonic classifiers has advantages, such as high efficiency, ease of maintenance and ease of cleaning. Small cyclones, e.g. having a diameter of less than 1 metre, typically have the highest efficiency. The use of a cyclonic classifier is especially suitable for accurately separating different lime particle sizes on an industrial scale. Other suitable processes for preparing fractions of particulate lime having particle sizes as disclosed herein include sieving and screening. For example, mechanical vibratory sieves (also referred to as gyratory separators or screening machines) are conventional in processing dry bulk powders and classify materials by separating them by particle size through a screen mesh. Using a combination of horizontal and vertical movements by means of a vibratory motor, they spread the material over a screen in controlled flow patterns and stratify the product. Different sieving techniques may be used to produce different particle size ranges of lime for use according to the invention.

Particle sizes and size distributions are defined herein according to the sizes of particles which will typically pass through, and/or be retained by, conventional sieves having a given mesh size. For example, a particle having a size of between 100 and 200 μm would pass though a 200 μm mesh sieve but would be retained by a 100 μm mesh sieve. Similarly, a particle having a size of less than 100 μm is capable of passing though a 100 μm mesh sieve and a particle with a size of more than 600 μm is capable of being retained by a 600 μm mesh sieve. It would be understood that particles are typically anisotropic and non-uniform and that sieving relates primarily to the maximal diameter of any particle, but that it is not exclusive, e.g. in the case of partially uniform shapes such as cylinders. Apparatus for sieving and separating particulates of the sizes defined herein is known, e.g. screens and sieves produced by Fredrik Mogensen AB (Sweden), Retsch AS (Norway), and Retsch GmbH (Germany).

Particulate lime according to the present invention is generally in a form which is active when contacted with the water in which fish and/or their ectoparasites are housed. By "active" is meant that the lime is capable of killing a significant proportion (e.g. at least 20%, 30%, 40% or 50%) of ectoparasites attached to a fish treated with the lime but without significantly increasing mortality in the treated fish (e.g. wherein fish mortality is below 10%, 5%, 2% or 1%). Preferably the lime particles are of a size which is not too small to get lodged in the gills of the fish but which is not too large to sediment immediately.

Preferably, the particulate lime of the invention has a particle size (when measured by sieving) of greater than about 10 µm, especially greater than about 20, 50, 75, 100, 120 or 200 µm. The particulate lime of the invention preferable has a particle size of less than about 2 mm, especially less than about 1 mm, or less than about 500, 400, 300, 200, 100 or 50 µm. Especially preferably, the particulate lime of the invention has a particle size of up to 4 mm, especially between about 10 µm and about 2 mm, particularly between about 20 µm and about 1 mm. Especially preferably, the particulate lime of the invention has a particle size in the range of about 50-800 µm, preferably about 75-600, about 100-400, about 120-300 or about 150-250 µm, e.g. about 100-200 µm.

In a further preferred embodiment, fewer than 10% of the particles (by mass) have a particle size below 10 µm, especially whereby fewer than 10% of the particles (by mass) have a particle size below 20, 50, 80 or 100 µm, or below 0.2, 0.5 or 1 mm. Especially preferably fewer than 10% of the particles (by mass) have a particle size below about 67 µm. It is also preferred that fewer than 10% of the particles (by mass) have a particle size above 5 mm, especially whereby fewer than 20% of the particles have a particle size above 4, 3, 2, 1.5, 1, 0.5, 0.4 or 0.2 mm. Especially preferably, fewer than 10% of the particles (by mass) have a particle size above about 370 µm.

Especially preferably, the particulate lime of the invention comprises a major fraction, e.g. at least about 50%, 75%, 80%, 90%, 95%, 98% or 99% by mass, of particles having a size in the range of about 10-1000 µm, especially about 100-600 µm, e.g. in the range of about 100-200, 200-300 or 300-400 or 400-600 µm. In an especially preferred embodiment, the particulate lime of the invention (e.g. quicklime) comprises a distribution of particle sizes such that no more than 10% of the particles (by mass) have a size of less than about 67 µm, not more than 10% of the particles (by mass) have a size of more than about 370 µm, and 50% of the particles have a size of at least about 176 µm. Preferably, the particulate lime has a size distribution substantially as illustrated in Figure 1.

The particulate lime of the invention is typically contacted with the aqueous environment (e.g. the water) housing the fish and/or their ectoparasites by sprinkling the lime on the surface thereof or by suspension of the particles in a liquid carrier (especially fresh water or salt water, e.g. seawater) and addition of the suspension to the aqueous environment.

The lime is preferably added in an amount of at least about 0.01 kg/m$^2$ of water surface, especially in an amount of at least about 0.02, 0.05, 0.1, 0.15, 0.2, 0.25, 0.3 or 0.5 kg/m$^2$ of water surface. Preferably the amount of lime added is less than about 2 kg/m$^2$ of water surface, especially less than about 1.5, 1, 0.75, 0.5, 0.3, 0.25 or 0.2 kg/m$^2$ of water surface. Particularly preferably, the lime is added in an amount between 0.01 and 2 kg/m$^2$ of water surface, especially between 0.05 and 1 kg/m$^2$ or between 0.1 and 0.5 kg/m$^2$ of water surface, e.g. about 0.1, 0.15, 0.2, 0.25, 0.3 or 0.5 kg/m$^2$ of water surface.

Where the aqueous environment housing the fish is well defined in volume of water (e.g. in the case of a tank, cage or other substantially fixed structure enclosing the fish), the lime is preferably added in an amount of at least 10 g/m$^3$ water, especially in an amount of at least about 20, 50, 100, 150, 200, 250, 300 or 500 g/m$^3$ water. Preferably the amount of lime added is less than about 2000 g/m$^3$ water, especially less than about 1500, 1000, 750, 500, 300, 250 or 200 g/m$^3$ water. Particularly preferably, the lime is added in an amount between 10 and 2000 g/m$^3$ water, especially between 50 and 1000 g/m$^3$ water or between 100 and 500 g/m$^3$ water, e.g. about 100, 150, 200, 250, 300 or 500 g/m$^3$ water.

In a further embodiment, especially where large numbers of fish are being treated, it is preferred that the lime is added in an amount of between 1 and 5 kg per 1000 fish, especially between 2 and 4 kg, e.g. about 3 kg, per 1000 fish. For example, in an industrial setting, treating with 3 kg of lime per 1000 fish in a typical 25 m by 35 m pen housing 100,000 fish would correspond to a dose 300 kg of lime.

In one embodiment of the invention, the lime is administered in a single dose. In an alternative embodiment, the lime is administered in multiple doses, e.g. 2, 3, 4 or more than 4 doses and generally at a time interval of between 1 minute and 24 hours, e.g. between 30 minutes and 12 hours, e.g. approximately hourly. Preferably the lime is administered in two doses at a time interval of about 30 minutes. The total dosage added in a 24 hour period may be an amount as defined above, e.g. the total dosage added over a 24 hour period is preferably less than about

2, 1, 0.5 or 0.3 kg/m$^2$ of water surface. Alternatively, each dosage may be an amount as defined above, e.g. each dosage is preferably an amount of lime between 10 and 2000 g/m$^3$ water, e.g. about 300 g/m$^3$ water.

Preferably, the lime is administered at a time other than a feeding time, i.e. the lime is not administered at the same time as fish food is added to the water. Especially preferably, the lime is added shortly after the fish have finished feeding, e.g. around 15, 30, 60 or 120 minutes after food has been added to the water. Alternatively, the fish are not fed during the treatment period, e.g. for a period lasting for up to 3 days, e.g. about 48 or 24 hours.

The lime treatment may be administered to water of varying salinities. Typically, the water treated may be fresh water (in the case of freshwater fish) or sea water (in the case of seawater fish). Water of an intermediate salinity may be treated where the fish are, for example, being acclimatised from a fresh water to a sea water regime or visa versa. Water having a salinity between 0 and 50 parts per thousand, e.g. between 0.5 and 30 parts per thousand, may typically be treated.

The lime treatments of the invention may be practised at a range of temperatures. Typically, the treatments are carried out *in situ*, e.g. in coastal, river, lake or estuary waters, at the ambient temperature. Preferred temperatures are between 0 and 25 ºC, especially between 1 and 20 ºC, between 2 and 15 ºC or between 4 and 10 ºC, e.g. around 5 ºC or around 10 ºC. Preferably the temperature is at least about 6 ºC, e.g. between 6 and 10 ºC. When the particulate lime comprises calcium oxide the treatment will, by nature of the reaction between the lime and water, heat the treated water somewhat and it is preferred that the body of water housing the fish (e.g. per m$^3$ of water) is heated by no more than 0.5 ºC, especially by no more than 0.2 or 0.1 ºC by the lime treatment.

The treatments of the invention are typically carried out in seawater or fresh water under the usual conditions for raising the fish species in question. For example, the pH of the water (especially fresh water) may be between 6 and 9, e.g. between 6 and 8, such as around 7.5 and the alkalinity may be >20 mg/L (expressed as the concentration of $CaCO_3$), e.g. between 20 and 300 mg/L.

The particulate lime treatments (e.g. the methods) of the invention may effectively be carried out without the addition of further anti-parasitic agents.

Accordingly, in a preferred embodiment, the particulate lime treatments of the invention are carried out without the addition of further anti-parasitic agents. However, in one embodiment, the invention provides methods as herein described wherein the particulate lime is administered (e.g. contacted with the aqueous environment as described herein) in combination with one or more further anti-parasitic agents. Said further anti-parasitic agents may be administered separately, sequentially or substantially simultaneously, e.g. shortly before or shortly after treatment with the particulate lime. The further anti-parasitic agents may be administered in their convention manner, e.g. by introduction into the water of the aqueous environment or by incorporation into administered fish food. In one embodiment, the one or more further anti-parasitic agents are bathing agents selected from: organophosphates (e.g. azamethiphos and dichlorvos); pyrethroids (e.g. deltamethrin and cypermethrin); carbamates (e.g. carbaryl); and/or in-feed agents such as avermectins (e.g. emamectin benzoate). In the embodiment where the particulate lime is administered in combination with an in-feed agent, the in-feed agent is preferably administered in a fish food shortly before lime treatment, e.g. around 15, 30, 60 or 120 minutes before lime treatment. The in-feed agent is typically administered in the fish food in multiple doses, e.g. across a period of at least 1 day, e.g. between 2 and 14 days, especially around 7 days.

In a further aspect, the invention provides the use of particulate lime in the methods of the invention. Particularly, the invention provides the use of particulate lime as defined herein in a method of treating an ectoparasitic infection in a fish, in a method of treating the aqueous environment of a fish farm to kill fish ectoparasites in said environment, or in a method of killing fish ectoparasites.

In one embodiment, the methods and uses of the invention involve the application of lime in combination with one or more other components. The lime may be combined with other components by co-formulation (i.e. in particles comprising the lime and the other components together) or by admixture. The other components include further anti-parasitic agents, e.g. as discussed above, and inert materials, referred to herein as "fillers". By "inert" is meant that the filler does not have any significant anti-parasitic effect when applied alone. The presence of the inert filler in combination, e.g. admixture, with the lime can be beneficial. For example, fillers may be used as diluents, to stabilise the particulate lime, to improve the flow properties (i.e. the flowability) of the particulate lime, and/or to control clouding (e.g. to reduce the risks associated with handling finely-ground lime material).

Examples of inert fillers which may be used in admixture with particulate lime as defined herein include calcium carbonate, sand, clay, mica, silica, talc and the like. Such materials are typically widely available in a variety of forms. Where an inert filler is used, this is preferably present in particulate form, especially having a particle size as described herein for the particulate lime. Particularly preferably the inert filler has a particle size which is large enough to prevent the filler becoming lodged in the gills of the fish to which the lime-containing admixture is to be administered. In a further preferred embodiment the inert filler is substantially free of water, e.g. having a level of residual water low enough that no significant reaction takes place with the lime (especially where the lime is quicklime) when the two are admixed. The inert filler therefore preferably contains less than 15% by weight of residual water, preferably less than 10%, 7.5% or 5% by weight of residual water. Methods for reducing the water level of inert fillers to provide substantially water-free materials are known in the art such as heating in an oven to constant weight (e.g. for around 7 hours).

The amount of inert filler to be mixed with the lime may vary depending on the nature of the filler, the characteristics required, etc. However, the inert filler is preferably added in an amount of up to 400% by weight of the lime, especially between 5 and 200%, between 10 and 100%, or between 20 and 50%, e.g. around 30% by weight of the lime.

Thus, in a related aspect the invention provides particulate compositions comprising lime, as well as the use of particulate compositions comprising lime in the methods herein defined. The particulate composition preferably comprises lime in combination with another component, e.g. an inert filler. The size of the particles in the particulate compositions is preferably as hereinbefore defined, e.g. between 100 and 600 μm, especially between 120 and 300 μm.

In another aspect, the invention provides a composition comprising (e.g. consisting essentially of) particulate lime for use in the methods of the invention. In one embodiment the composition comprises (e.g. consists essentially of) particulate lime and one or more inert fillers as defined herein. As would be understood, particulate lime as defined herein is suitable for use in methods of the invention and suitable compositions are not limited to any specific examples of particulate lime.

In a related aspect, the invention provides a composition comprising particulate lime for use in a method (e.g. a therapeutic method) of treating an ectoparasitic infection of a fish, e.g. wherein said method comprises contacting water housing said fish with particulate lime in a sufficient quantity to remove and/or kill the ectoparasites. Also provided is the use of particulate lime in the preparation of a medicament for use in a method of treating an ectoparasitic infection in a fish. The medicament may be a composition as herein defined.

In a further aspect, the invention provides a composition (especially an ectoparasitisidic composition, e.g. a delousing composition) comprising particulate lime, optionally in combination with one or more further anti-parasitic agents. Said further parasitic agents are preferably as herein defined, especially anti-parasitic bathing agents as herein defined. Said composition optionally further comprises one or more inert fillers as herein defined. Compositions in accordance with the invention may have any of the characteristics hereinbefore defined, e.g. at least 90% by weight of CaO and/or $Ca(OH)_2$.

Especially preferably, the methods, uses and compositions described herein are directed to the treatment of sea lice (e.g. *L. salmonis*) on salmon (especially farmed Atlantic salmon) in seawater.

Alongside the observed antiparasitic effects of the particulate lime of the invention, the inventors have also found that treatment of the aqueous environment of farmed fish, e.g. salmon in net pens or cages, with particulate lime (e.g. CaO) is capable of killing filamentous algae and hydroids in the aqueous environment of the pens. Clogging of net pens with marine organisms can reduce water quality inside the pens and removing the biomass is a laborious and costly operation. For example, the Norwegian fish farming industry is particularly affected by hydroid fouling (*T. larynx*) between July and November, during which period fish farmers may need to clean their nets every 2 weeks. Use of particulate lime as defined herein provides means to reduce biofouling of net pens and cages whilst the fish are *in situ* and therefore offers a cheap and practical alternative to known methods. Reduction of biofouling can be achieved in addition to, or alongside, treatment of fish for ectoparasitic infections as described herein.

In this aspect, the invention therefore provides a method for reducing biofouling on a sub-water-surface structure, the method comprising contacting the aqueous environment of said structure with particulate lime, preferably wherein said aqueous environment houses fish (e.g. the fish species mentioned herein).

In a related aspect, the invention provides a method for improving the quality of water in and/or around a sub-water-surface structure which structure carries at least one attached marine organism that is capable of reducing the quality of said water, the method comprising contacting the aqueous environment of said structure with particulate lime, preferably wherein said aqueous environment houses fish (e.g. the fish species mentioned herein).

In a further related aspect, the invention provides the use of particulate lime for reducing biofouling on a sub-water-surface structure, and/or for improving the quality of water in and/or around a sub-water-surface structure which structure comprises at least one attached marine organism that is capable of reducing the quality of said water.

In a preferred embodiment, the fish is a farmed fish, especially a farmed salmonid fish such as an Atlantic salmon. The sub-water-surface structure is preferably a net, pen, cage, or the like, which houses said fish. The lime used in the above methods is particulate lime as defined herein.

The methods are preferably directed against biofouling caused by a permanent or semi-permanent marine organism, e.g. hydroids and/or algae, especially filamentous algae. The attached marine organism that is capable of reducing the quality of water is also preferably a hydroid or algae, especially filamentous algae. Preferred filamentous algae to target according to the invention include *Enteromorpha clathrata* and *Enteromorpha intestinalis*. Preferred hydroids to target according to the invention include those of the genus *Tubularia*, especially *T. larynx* (also called *Ectopleura larynx*).

In a further embodiment of the invention, administration of particulate lime may be performed in conjunction with treatment using cleaning fish (e.g. cleaner wrasse of the family *Labridae*). Treatment with cleaner fish is known to reduce the levels of ectoparasites, e.g. salmon lice, on infected fish but the cleaner fish generally do not perform efficiently in enclosures (e.g. tanks, cages or nets) which are fouled with marine biomass such as filamentous algae. Since the particulate lime treatment of the invention can clean bio-fouled enclosures, the treatment can increase the efficiency of the cleaner fish whilst simultaneously killing and/or detaching a proportion of the ectoparasites from the infected fish. Particulate lime may be added before or during treatment of infected fish with cleaner fish.

Thus, in a preferred embodiment of the methods of the invention, the fish (being capable of infection by the ectoparasites) are treated with cleaner fish (e.g. wrasse) during or after particulate lime treatment

The use of lime according to the present invention also enables the treatment of farmed fish, e.g. according to the methods described herein, without the use of the barrier or skirt that would typically be placed around the fish net or cage when conventional antiparasitic agents are administered. This has the advantages of lower costs and a lesser degree of stress to the fish.

In one embodiment, especially where the fish are housed in an enclosure which experiences significant water movement (e.g. sea or river currents), the enclosure (e.g. a net or cage) housing the farmed fish may be partially or totally enclosed by an impermeable barrier. However, it is preferred that the enclosure housing the farmed fish is not partially or totally enclosed by an impermeable barrier. Thus, in a preferred embodiment of the methods of the invention, said fish (being capable of infection by the ectoparasites) are housed in an enclosure, wherein said enclosure is not partially or totally surrounded by an impermeable barrier, e.g. a tarpaulin.

The invention will now be illustrated with reference to the following non-limiting Examples and Figures in which:

Figure 1 shows the particle size distribution of the particulate quicklime produced according to Example 8.

Example 1 - Size distribution of lime particles

Particulate lime (predominantly calcium oxide) having particle sizes between 0 and 2 mm was obtained from Franzefoss Miljøkalk AS (Miljø Brentkalk 0/2). 400 g of the particulate material was fractionated by sieving using a standard laboratory machine sieve with graduated mesh sizes of between 1250 µm and 100 µm (Retsch AS 200 with Retsch GmbH stainless steel sieves). The mass of particles in the various fractions was weighed and a size distribution calculated based on the amount of material recovered in each size range. A total of 99.3% of the initial material was recovered. The distribution of particle sizes is shown in Table 1 below.

*Table 1 - Amount of particulate lime in each size fraction*

| Particle size range (µm) | Mass (g) | % of material recovered |
|---|---|---|
| | | |
| 0-100 | 71.2 | 17.9 |
| 100-200 | 166.2 | 41.9 |
| 200-300 | 83.9 | 21.1 |
| 300-400 | 30.0 | 7.6 |
| 400-600 | 23.5 | 5.9 |
| 600-900 | 9.5 | 2.4 |
| 900-1255 | 5.7 | 1.4 |
| ≥1250 | 7.0 | 1.8 |
| | | |
| Total: | 397.0 | 100.0 |

The data in Table 1 show that the commercially-available material contains particles which are predominantly less than 300 µm in size (as measured by their ability to pass through a 300 µm mesh sieve) and that a significant amount of the particles present, around 20% by mass, are less than 100 µm in size.

Example 2 - Preparing fractions of lime particles for testing

Fractions of particulate lime having sizes in the range 0-600 µm were prepared as described in Example 1. Fractions having sizes in the range of 200-300, 300-400 and 400-600 µm were combined and mixed to provide a single fraction with particles having sizes between 200-600 µm.

The three separate fractions, 0-100 µm, 100-200 µm and 200-600 µm, were stored under substantially anhydrous conditions before being used.

Example 3 - The effect of particulate lime of salmon lice

Salmon lice (*Lepeophtheirus salmonis*) were carefully scraped off five anaesthetised Atlantic salmon (*Salmo salar*) using thin plastic foil pieces measuring approximately 10x5 cm and placed into five containers, each containing 0.5 l of seawater, the containers having dimensions of 18 x 12 x 6 cm and a surface area of 216 $cm^2$.

Different amounts of particulate lime (0-2 mm commercial sample as described in Example 1) were sprinkled on the surface of the water and the containers were refrigerated at approximately 5 °C. Mortality was measured by observation after 24 hours. Table 2 shows the results of this experiment.

*Table 2 - The effect of particulate lime on L. salmonis*

| Concentration ($kg/m^2$) | Total number of lice | Mortality (%) |
|---|---|---|
| | | |
| 0 | 17 | 0 |
| 0.05 | 10 | 80 |
| 0.1 | 8 | 87.5 |
| 0.15 | 7 | 100 |
| 0.2 | 7 | 100 |

The data in table 2 demonstrate that sea lice can be killed effectively using relatively low amounts (0.05 $kg/m^2$ or 0.5 $g/cm^2$) of particulate lime sprinkled on the surface of water housing the lice.

Example 4 - The effect of particulate lime versus lime milk

Three experimental tanks were set up. Each tank had a volume of approximately 1000 litres of sea water and housed 11 trout (*Oncorhynchus mykiss*). The water temperature of each tank was approximately 6 °C.

Lime milk was prepared by dissolving 300 g of CaO (the 0-2 mm commercial sample of CaO described in Example 1) in 4 litres of fresh water, mixing it for 15 minutes, and leaving it to stand for another 15 minutes before mixing it again. A fine, milky solution with no visible particles was obtained which was barely warmer than the temperature of the starting water.

In the first tank, the 4 litre solution of lime milk was added to the tank water housing the fish and mixed using a circulation pump; a second 4 litre solution of lime milk was added after 30 minutes. No settling of particles onto the backs of the fish was observed.

In the second tank, a first dose of 300 g particulate lime (the 0-2 mm commercial sample of CaO described in Example 1) was added immediately after mixing it with 4 litres of ambient temperature seawater (no reaction was observed during this pre-treatment step due to the use of seawater at low temperature and the very short pre-treatment time). A further 300 g of particulate lime was added in the same way 30 minutes later. A lot of the lime particles settled on the back and sides of the fish.

The third tank was untreated and acted as a control. 18 hours after first addition of the lime or lime milk, the number of live lice on the trout was counted and the level of fish mortality was also determined. Table 3 shows the results from this experiment with values calculated relative to the control.

*Table 3 - Effect of treating infested fish with lime milk and particulate lime*

| Treatment | Reduction in lice (%) | Fish mortality per tank |
|---|---|---|
| | | |
| Control | - | 0 of 11 |
| Lime milk | 25 | 9 of 11 |
| Particulate lime | 38 | 0 of 11 |

The data in Table 3 demonstrate that trout are sensitive to lime milk (fine $Ca(OH)_2$ particles) but are not sensitive to particulate lime treatment (CaO). In contrast, the fish lice are more sensitive to the particulate lime than to the lime milk with nearly a 40% reduction in infestation observed for trout treated with the particulate lime.

Example 5 - The effect of the dosage of particulate lime

In this experiment, commercially-available particulate lime (CaO) of size 0-2 mm as described in Example 1 was administered at moderate levels, the number of doses being varied from zero (the control) through one, two and three doses.

*Background experimental details*

Approximately 1000 (average of 200 g) salmon (*Salmo salar*) were transferred to a 5 x 5 x 10 m net pen in February. The louse status of the fish was monitored regularly until start of the experiments in June of the same year.

Fish were transferred from the net pen to 1000 litre closed on-shore containers by use of a landing net containing water. Temperature in the net pen was 10 °C at a 5 m depth. Thereafter, the fish were moved from the container to the experimental tanks by use of a landing net. Experimental tanks received water from 160 m depth that varied in temperature from 8.2 to 8.6 °C during the experimental period. No sedative chemicals were used in the operations.

The experimental tanks were cylindrical (1.5 m diameter x 0.9 m height). Water flow was 40 litre/min in each tank, creating a circular movement of water and thereby a uniform positioning of the fishes.

Experimental treatment started approximately two hours after distribution of the fish to the experimental tanks was finished. Duplicate tanks were used for each treatment and triplicate tanks for the control.

*Specific experimental details*

All treatment tanks received their first dose of particulate lime at start up, at the fourth hour in the two-application group, and at the second and fourth hours in the three-application group. The number of lice was measured three days after start-up.

One "dose" of particulate lime was 353 g, which corresponds to 0.2 $kg/m^2$ of water surface. There were between 17 and 19 fishes in each tank. Three untreated tanks were used as controls. Two tanks were used for each treatment.

32 fish were sub-sampled from the transport container at start up, weighed and examined for lice. The initial average fish weight was 513 g and average lice number was 7.0 per fish, of which 11% were mature female lice.

Egg strings from one treated and one control group were sampled in 200 ml containers. Approximately 8 egg strings were removed from the lice by use of scissor and forceps, from each treatment. The containers were stored in a refrigerator for four days.

*Results*

Over the experimental period, the number of lice per fish in the control tank dropped from an average of 7.0 to 4.9. The latter number was used as basis for calculating the effect of different treatments.

After applying the first dose at start-up, the fish where observed to be covered in white CaO powder. The white cover gradually disappeared during the experimental period and, after 3 days, the 1- and 2-dose application groups had lost all their white cover, while it was still observable in the 3-dose application group. Treated fish appeared sluggish at the beginning of the experiment and did not show avoidance behaviour when the experimenter approached the fish tank. The fish gradually recovered during the experimental period and appeared similar to control fish in behaviour and appearance at the end of the experiment. Data are shown in Figure 4, below.

Salmon lice reduction was not correlated with number of applications, approximating 60% for all three treatments. However, mortality was strongly correlated with number of applications, ranging from 3% to 30%. The treatments also appear to kill fewer mature female lice than other categories, as the fraction of mature lice increased from 16% in the control to more than 30% in the treatment groups. Although no systematic observations on salmon lice distribution on the fish was conducted, it was observed that a relatively large fraction of the mature female lice alive in the treatment groups gathered on the ventral side of the fish, just anterior to the tail fin. This pattern was repeated in the experiment described in Example 6.

*Table 4 - Effect of the number of doses on salmon lice and fish mortality*

| Treatment | Reduction in lice (%) | Mature females (%) | Fish mortality (%) |
|---|---|---|---|
| | | | |
| Control | - | 16 | 0 |
| 1 dose | 59 | 33 | 3 |
| 2 doses | 64 | 31 | 8 |
| 3 doses | 63 | 37 | 30 |

When hatching of egg strings were evaluated, far more nauplii (free-swimming larvae) were observed in the untreated group compared to the treated group, indicating that treatment with particulate lime (CaO) had impaired the hatchability of salmon lice eggs.

Example 6 - Effect of particle size on lice and fish

The aim of this experiment was to test the effect of different sizes of the particulate lime on lice numbers and fish mortality. The background experimental details were identical to Example 5.

*Specific experimental details*

Four treatment regimes were employed; one control group (no treatment) and three treatment groups using the three different particulate lime fractions described in Example 2. Results were obtained two days after start-up.

There were 23 to 26 fishes in each tank and two tanks were used for each lime treatment. All tanks were given three treatments: 100 g at start-up, 100 g after 3 hours and 50 g after 29 hours. These treatments correspond to approximately 0.05, 0.05 and 0.03 kg/m$^2$ of water surface of particulate lime, respectively.

17 fish were sub-sampled from the net pen at start-up, weighed and examined for lice. The initial average weight was 602 grams and average lice number was 7.8 per fish, of which 16% were mature females.

*Results*

A few minutes after applying the first dose (100 g) it was observed that the fish in tanks receiving the 100-200 μm and 200-600 μm fractions were covered in white powder (CaO), while the fish treated with the 0-100 μm fraction did not have an observable covering. When the second dose was applied after 3 hours, all of the fish in the tanks that had received the 0-100 μm fractions were swimming close to the water surface, while the other fish where fairly evenly distributed in the tanks. This trend was not as pronounced during the rest of the experiment. At the end of the experiment, the fish in the different treatments were easy to distinguish visually because there was no observable powder on the fish in the tanks treated with the 0-100 μm fraction, a reduced but distinct cover in the 200-600 μm fraction groups, and a intermediate cover of the fish in the 100-200 μm fraction groups.

Reducing the doses compared to the experiment described in Example 5 had a strong effect on fish mortality, as only 1 fish died in this experiment (it was not determined whether the particulate lime was responsible for this isolated event). Particle size had a significant effect on lice reduction, with only a 15% reduction being measured in the 0-100 μm fraction group, while this number was three times higher for the two other treatments. Also, in the 0-100 μm fraction group there was no increase in the proportion of mature females, whereas this fraction was more than doubled in the two other groups. The results are shown in Table 5 below.

*Table 5 - The effect of particle sizes on salmon lice and fish mortality*

| Treatment | Reduction in lice (%) | Mature females (%) | Fish mortality (%) |
|---|---|---|---|
| | | | |
| Control | - | 16 | 0 |
| 0-100 μm | 15 | 15 | 0 |
| 100-200 μm | 54 | 35 | 2 |
| 200-600 μm | 43 | 38 | 0 |

These data show that even at low dosages, particulate lime is capable of killing and/or detaching a significant proportion of fish lice. Particles of an intermediate size, e.g. between 100 and 600 µm, especially 100-200 µm, are especially potent and yield excellent results with little or no long-term effects on the treated fish.

Example 7 - use of particulate lime for removing ectoparasites and cleaning enclosures

*Experimental details*

The net cage described in Example 5 (5x5 m surface area) was left for a time to allow biofouling by aquatic epiphytes to occur. The net, housing over 500 fish (*S. salar* weighing approximately 600 grams on average) was then treated with particulate lime (a 100-600µm fraction being a mixture of the 100-200 and 200-600 µm fractions of Example 2). The water temperature was approximately 10 °C.

Before treating the fish, the depth of the net was reduced to approximately 2 m by lifting the sides and dropping the excess net back into the sea on the back side of the net containing the fish. The fish were gently transferred to one half of the cage by lining of the other half. Then 1.6 kg of the lime was sprinkled on the cage surface at time 0, 24 and 48 hours. After each treatment the net was dropped back into the water to restore the full volume of enclosure.

A sample of 17 fish was taken before the experiment started and the total number of lice was measured on each fish as described above. 48 hours after the last treatment, a sample of 14 different fish was taken and salmon lice were counted. The level of biofouling on the net cage was assessed by visual observation.

*Results*

The fish did not appear stressed during the treatments and no fish mortality was recorded during or after the lime treatment.

The reduction in average number of lice per fish was from 7.8 at the start of the experiment to 1.5 after 96 hours. This equates to an 81% reduction in fish lice. In addition, the net cage was cleaned of epiphytes.

Example 8 - Production and characteristics of a particulate lime product

A lime particulate (quicklime) was produced by Franzefoss Miljøkalk AS (Rud, Norway) by burning limestone ($CaCO_3$) mined from Tromsdalen in Verdal (Verdalskalk AS, Norway) in an oven at 1000 °C. The lime was screened, sized and/or crushed before being separated by an air classifier to provide the material characterised in Table 6, below. This product is not believed to be available commercially.

*Table 6 - Characteristics of the lime product*

| Parameters | Analysis method | Values | | Units |
|---|---|---|---|---|
| | | (average) | (s) | |
| | | | | |
| *Components* | | | | |
| CaO (active) | Wet chemical | 92 | 1.3 | [%] |
| $CO_2$ (residual) | | 2 | 0.5 | [%] |
| CaO (total) | WD-XRF | 96 | 0.7 | [%] |
| MgO | | 0.7 | 0.02 | [%] |
| $SiO_2$ | | 0.6 | 0.13 | [%] |
| $Al2O_3$ | | 1 | 0.2 | [%] |
| $Fe_2O_3$ | | 0.09 | 0.010 | [%] |
| MnO | | 0.005 | 0.0005 | [%] |
| $K_2O$ | | 0.048 | 0.0043 | [%] |
| $SO_3$ | | 0.20 | 0.023 | [%] |
| | | | | |
| *Elemental analysis* | | | | |
| Cl | Wet chemical | <0.005 | 0.0 | [%] |
| As | AAS | 1 | 0.0 | [ppm] |
| Pb | | 2 | 0.4 | [ppm] |
| Cd | | 0.08 | 0.045 | [ppm] |
| Hg | | 0.01 | 0.0 | [ppm] |
| B | ICP-AES | 11.4 | 3.4 | [ppm] |
| Cu | | 0.9 | 0.33 | [ppm] |
| Na | | <200 | 0 | [ppm] |
| Zn | | 52 | 8.4 | [ppm] |
| Ni | | 1.9 | 0.91 | [ppm] |
| Cr | | 0.6 | 0.06 | [ppm] |
| Se | | 1 | - | [ppm] |
| | | | | |

| *Physical and chemical analysis* | | | | |
|---|---|---|---|---|
| T (60 °C) | Reactivity | 89 | 29.0 | [seconds] |
| dT (total) | | 55 | 0.7 | [°C] |
| Mass density | Pycnometer | 3.32 | 0.05 | [kg/dm³] |
| 0.010 mm | Microtrack S 3501 | 3 | - | [%] |
| 0.063 mm | | 9 | - | [%] |
| 0.100 mm | | 21 | - | [%] |
| 0.125 mm | | 31 | - | [%] |
| 0.150 mm | | 40 | - | [%] |
| 0.600 mm | | 97 | - | [%] |
| d10% | | 67 | - | [μm] |
| d50% | | 176 | - | [μm] |
| d90% | | 370 | - | [μm] |

The particle size distribution of the classified material was analysed and found to have the characteristics shown in Figure 1.

Claims:

1. Use of particulate lime which is a calcium-containing inorganic material which comprises calcium oxide and/or calcium hydroxide for treating an ectoparasitic infection in fish having ectoparasites, wherein water housing said fish is contacted with said particulate lime in a sufficient quantity to detach and/or kill the ectoparasites, and wherein said particulate lime comprises at least 90% by mass of particles having a size in the range of 10-1000 μm when measured by sieving.

2. Use of particulate lime which is a calcium-containing inorganic material which comprises calcium oxide and/or calcium hydroxide for treating the aqueous environment of a fish farm to kill fish ectoparasites in said environment, wherein water of the aqueous environment is contacted with said particulate lime, wherein said water houses farmed fish, and wherein said particulate lime comprises at least 90% by mass of particles having a size in the range of 10-1000 μm when measured by sieving.

3. Use of particulate lime which is a calcium-containing inorganic material which comprises calcium oxide and/or calcium hydroxide for killing fish ectoparasites, wherein water housing said ectoparasites is contacted with said particulate lime, wherein said water further houses farmed fish which are susceptible to infection by said ectoparasites, and wherein said particulate lime comprises at least 90% by mass of particles having a size in the range of 10-1000 μm when measured by sieving.

4. The use of any one of claims 1 to 3, wherein said ectoparasites are sea lice of the genera *Lepeophtheirus* and/or *Caligus*.

5. The use of claim 4, wherein said ectoparasites are *Lepeophtheirus salmonis*, *Caligus rogercresseyi*, *Caligus clemensi*, *Caligus chiastos*, *Caligus epidemicus* and/or *Caligus elongates*.

6. The use of claim 4, wherein said ectoparasites are *Lepeophtheirus salmonis*.

7. The use of any one of claims 1 to 6, wherein said fish is a salmonid fish.

8. The use of claim 7, wherein said fish is an Atlantic salmon (*Salmo salar*) or rainbow trout (*Oncorhynchus mykiss*).

9. The use of any one of claims 1 to 8, wherein said particulate lime is at least 50% calcium oxide.

10. The use of claim 9, wherein said particulate lime is at least 95% calcium oxide.

11. The use of any one of claims 1 to 10, wherein said particulate lime has a particle size in the range of 100-200 or 200-600 μm when measured by sieving.

12. The use of any one of claims 1 to 10, wherein said particulate lime has a particle size in the range of 100-200 μm when measured by sieving.

13. The use of any one of claims 1 to 12, wherein the lime is in admixture with one or more inert fillers.

14. The use of claim 13, wherein the inert filler is a particulate material selected from the group consisting of calcium carbonate, sand, clay, mica, silica and talc.

15. The use of any one of claims 1 to 12, wherein the lime is co-formulated with at least one other component.

16. The use of claim 15, wherein the lime is co-formulated with at least one other inert filler selected from the group consisting of calcium carbonate, sand, clay, mica, silica and talc.

17. The use of any one of claims 1 to 16, wherein said particulate lime is contacted with the water housing the fish and/or their ectoparasites in an amount of at least 0.01 $kg/m^2$ of water surface.

18. The use of claim 17, wherein said particulate lime is contacted with the water housing the fish and/or their ectoparasites in an amount between 0.01 and 2 $kg/m^2$ of water surface.

19. The use of claim 17, wherein said particulate lime is contacted with the water housing the fish and/or their ectoparasites in an amount of 0.1, 0.2 or 0.3 $kg/m^2$ of water surface.

20. The use of any one of claims 1 to 16, wherein said particulate lime is contacted with the water housing the fish and/or their ectoparasites in an amount of at least 10 $g/m^3$ water.

21. The use of claim 20, wherein said particulate lime is contacted with the water housing the fish and/or their ectoparasites in an amount between 10 and 2000 $g/m^3$ water.

22. The use of claim 20, wherein said particulate lime is contacted with the water housing the fish and/or their ectoparasites in an amount of 100, 200 or 300 $g/m^3$ water.

23. The use of any one of claims 1 to 22, wherein said particulate lime is contacted with the aqueous environment or water housing the fish and/or their ectoparasites in a single dose or in multiple doses.

24. The use of claim 23, wherein said particulate lime is contacted with the aqueous environment housing the fish and/or their ectoparasites in 2, 3, 4 or more than 4 doses.

25. The use of claim 23 or claim 24, wherein said doses are administered at a time interval of between 30 minutes and 12 hours.

26. The use of any one of claims 1 to 25, wherein the total dosage of particulate lime added in a 24 hour period is less than 1 $kg/m^2$ of water surface.

27. The use of claim 26, wherein the total dosage of particulate lime added in a 24 hour period is less than 0.5 $kg/m^2$ of water surface.

28. The use of any one of claims 1 to 27, wherein said fish are treated with cleaner fish during or after particulate lime treatment.

29. The use of claim 28, wherein said cleaner fish are wrasse.

30. The use of any one of claims 1 to 29, wherein said fish are housed in an enclosure and wherein said enclosure is not partially or totally surrounded by an impermeable barrier.

31. A method of killing fish ectoparasites comprising contacting water housing said ectoparasites with particulate lime which is a calcium-containing inorganic material which

comprises calcium oxide and/or calcium hydroxide, wherein said particulate lime comprises at least 90% by mass of particles having a size in the range of 10-1000 μm when measured by sieving, and wherein said water housing said ectoparasites does not contain farmed fish which are a host for said ectoparasites.

32. The method of claim 31, wherein said ectoparasites are sea lice of the genera *Lepeophtheirus* and/or *Caligus*.

33. The method of claim 32, wherein said ectoparasites are *Lepeophtheirus salmonis*, *Caligus rogercresseyi*, *Caligus clemensi*, *Caligus chiastos*, *Caligus epidemicus* and/or *Caligus elongates*.

34. The method of claim 32, wherein said ectoparasites are *Lepeophtheirus salmonis*.

35. The method of any one of claims 31 to 34, wherein said particulate lime is at least 50% calcium oxide.

36. The method of claim 35, wherein said particulate lime is at least 95% calcium oxide.

37. The method of any one of claims 31 to 36 wherein said particulate lime has a particle size in the range of 100-200 or 200-600 μm when measured by sieving.

38. The method of any one of claims 31 to 36, wherein said particulate lime has a particle size in the range of 100-200 μm when measured by sieving.

39. The method of any one of claims 31 to 38, wherein the lime is in admixture with one or more inert fillers.

40. The method of claim 39, wherein the inert filler is a particulate material selected from the group consisting of calcium carbonate, sand, clay, mica, silica and talc.

41. The method of any one of claims 31 to 38, wherein the lime is co-formulated with at least one other component.

42. The method of claim 41, wherein the lime is co-formulated with at least one other inert filler selected from the group consisting of calcium carbonate, sand, clay, mica, silica and talc.

43. The method of any one of claims 31 to 42, wherein said particulate lime is contacted with the water housing the ectoparasites in an amount of at least 0.01 $kg/m^2$ of water surface.

44. The method of claim 43, wherein said particulate lime is contacted with the water housing the ectoparasites in an amount between 0.01 and 2 $kg/m^2$ of water surface.

45. The method of claim 43, wherein said particulate lime is contacted with the water housing the ectoparasites in an amount of 0.1, 0.2 or 0.3 $kg/m^2$ of water surface.

46. The method of any one of claims 31 to 42, wherein said particulate lime is contacted with the water housing the ectoparasites in an amount of at least 10 $g/m^3$ water.

47. The method of claim 46, wherein said particulate lime is contacted with the water housing the ectoparasites in an amount between 10 and 2000 $g/m^3$ water.

48. The method of claim 46, wherein said particulate lime is contacted with the water housing the ectoparasites in an amount of 100, 200 or 300 $g/m^3$ water.

49. The method of any one of claims 31 to 48, wherein said particulate lime is contacted with the water housing the ectoparasites in a single dose or in multiple doses.

50. The method of claim 49, wherein said particulate lime is contacted with the aqueous environment housing the ectoparasites in 2, 3, 4 or more than 4 doses.

51. The method of claim 49 or claim 50, wherein said doses are administered at a time interval of between 30 minutes and 12 hours.

52. The method of any one of claims 31 to 51, wherein the total dosage of particulate lime added in a 24 hour period is less than 1 $kg/m^2$ of water surface.

53. The method of claim 52, wherein the total dosage of particulate lime added in a 24 hour period is less than 0.5 $kg/m^2$ of water surface.

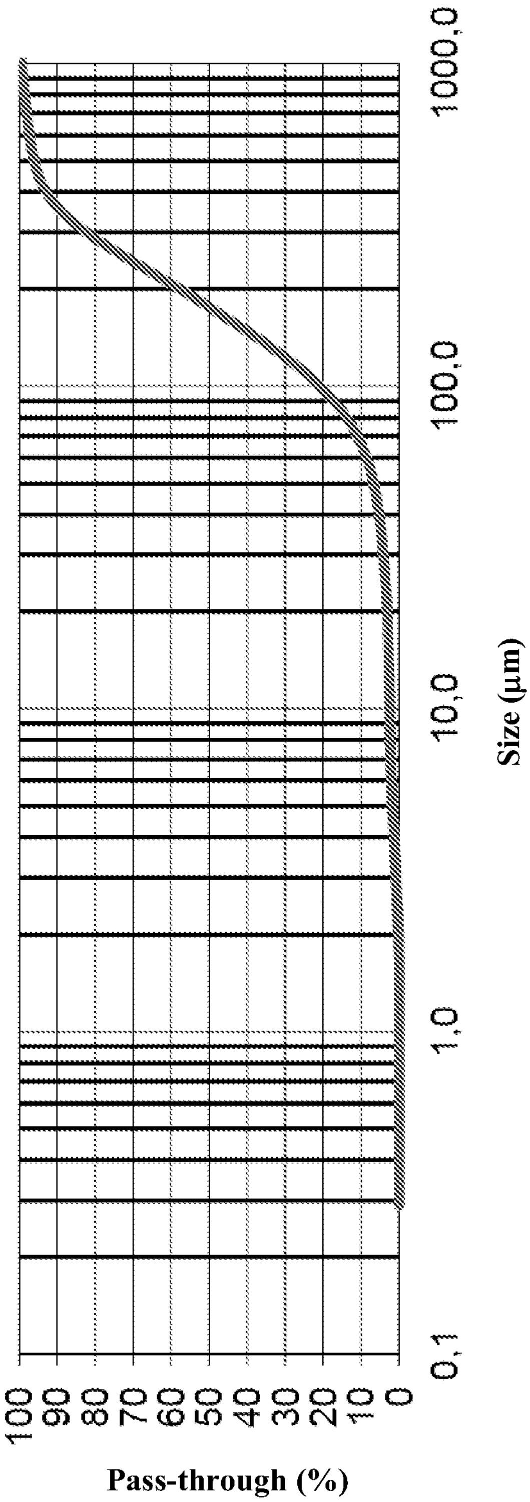
Size (µm)
0,1
1,0
10,0
100,0
1000,0
Pass-through (%)
100
90
80
70
60
50
40
30
20
10
0

FIG. 1