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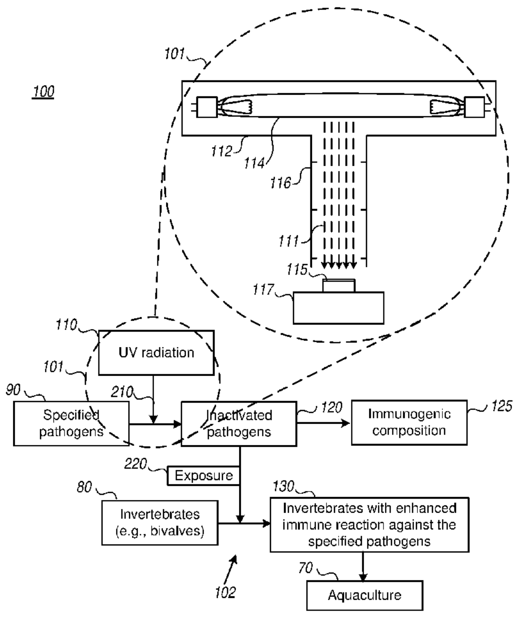

Figure 1A

(57) Abstract: Aquaculture systems and methods are provided, as well as immunogenic compositions and methods of immunologically protecting bivalves against specified pathogens. Methods include inactivating specified pathogens using UV (ultraviolet) radiation, exposing invertebrates grown or to be grown in aquaculture to the specified UV-inactivated pathogens to enhance an immune reaction of the exposed invertebrates toward the specified pathogens, and growing the exposed invertebrates in aquaculture. For example, the methods were demonstrated to increase the immune response of oysters to ostreid herpesvirus 1 (OsHV-1) following their prior exposure to UV-inactivated OsHV-1.

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IMMUNE-ENHANCED AQUACULTURE

BACKGROUND OF THE INVENTION

1. TECHNICAL FIELD

[0001] The present invention relates to the field of invertebrate aquaculture, and more particularly, to enhancing the immunity of grown invertebrate to pathogens.

2. DISCUSSION OF RELATED ART

[0002] Invertebrates grown in aquaculture are susceptible to a variety of waterborne pathogens. Bivalves, as filtering organisms, are especially susceptible. In contrast to vertebrates however, invertebrates lack an adaptive immune response and do not produce antibodies.

SUMMARY OF THE INVENTION

[0003] The following is a simplified summary providing an initial understanding of the invention. The summary does not necessarily identify key elements nor limit the scope of the invention, but merely serves as an introduction to the following description.

[0004] One aspect of the present invention provides a method comprising: inactivating specified pathogens using UV (ultraviolet) radiation, exposing invertebrates grown or to be grown in aquaculture to the specified UV-inactivated pathogens to enhance an immune reaction of the exposed invertebrates toward the specified pathogens, and growing the exposed invertebrates in aquaculture.

[0005] One aspect of the present invention provides an aquaculture system comprising: an immunization unit configured to expose invertebrates to specified UV-inactivated pathogens to enhance an immune reaction of the exposed invertebrates toward the specified pathogens, an aquaculture growth unit configured to grow the exposed invertebrates, and possibly a pathogen-inactivation unit configured to inactivate specified pathogens using UV radiation.

[0006] One aspect of the present invention provides an immunogenic composition comprising inactivated OsHV-1 (ostreid herpesvirus 1) and/or inactivated *Vibrio* bacteria, produced by irradiating the OsHV-1 viruses and/or the *Vibrio* bacteria with UV radiation.

[0007] One aspect of the present invention provides a method of immunologically protecting bivalves against specified pathogens, the method comprising irradiating the specified pathogens with UV radiation to yield an immunogenic composition, and exposing the bivalves to the immunogenic composition to enhance their immune reaction toward the specified pathogens.

[0008] These, additional, and/or other aspects and/or advantages of the present invention are set forth in the detailed description which follows; possibly inferable from the detailed description; and/or learnable by practice of the present invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0009] For a better understanding of embodiments of the invention and to show how the same may be carried into effect, reference will now be made, purely by way of example, to the accompanying drawings in which like numerals designate corresponding elements or sections throughout.

[0010] In the accompanying drawings:

[0011] Figures 1A, 1D and **1E** are high-level schematic block diagrams of aquaculture systems, according to some embodiments of the invention.

[0012] Figures 1B and **1C** are high-level schematic illustrations of pathogen-inactivation units, according to some embodiments of the invention.

[0013] Figure 2 is a high-level flowchart illustrating a method, according to some embodiments of the invention.

[0014] Figures 3A-3C illustrate the setting and results of experiments conducted to show the enhancing the immune response of *C. gigas* to OsHV-1 using UV-inactivated pathogens, according to some embodiments of the invention.

[0015] Figure 4 illustrates the effect of treatments (i) to (iv) on the expression of each of the eight genes presented in **Table 2**.

DETAILED DESCRIPTION OF THE INVENTION

[0016] In the following description, various aspects of the present invention are described. For purposes of explanation, specific configurations and details are set forth in order to provide a thorough understanding of the present invention. However, it will also be apparent to one skilled in the art that the present invention may be practiced without the specific details presented herein. Furthermore, well known features may have been omitted or simplified in order not to obscure the present invention. With specific reference to the drawings, it is stressed that the particulars shown are by way of example and for purposes of illustrative discussion of the present invention only, and are presented in the cause of providing what is believed to be the most useful and readily understood description of the principles and conceptual aspects of the invention. In this regard, no attempt is made to show structural details of the invention in more detail than is necessary for a fundamental understanding of the invention, the description taken with the drawings making apparent to those skilled in the art how the several forms of the invention may be embodied in practice.

[0017] Before at least one embodiment of the invention is explained in detail, it is to be understood that the invention is not limited in its application to the details of construction and the arrangement of the components set forth in the following description or illustrated in the drawings. The invention is applicable to other embodiments that may be practiced or carried out in various ways as well as to combinations of the disclosed embodiments. Also, it is to be understood that the phraseology and terminology employed herein are for the purpose of description and should not be regarded as limiting.

[0018] Embodiments of the present invention provide efficient and economical methods and mechanisms for improving the immunity of invertebrates against pathogens and thereby provide improvements to the technological field of aquaculture. Surprisingly, the it has been found that it is possible to inactivate pathogens and use them in vaccine-like approaches in invertebrates. For example, in bivalves, it was shown that the innate immune system of bivalves could be enhanced to develop a certain degree of memory, and consequently, immune protection against pathogen. As an example, UV radiation was used to inactivate important pathogens for the bivalve aquaculture industry, OsHV-1 (ostreid herpesvirus 1) as a virus representative and *Vibrio splendidus* as a bacterium representative, and have shown the inactivated pathogens to be immunostimulants in *Crassostrea gigas* (the Pacific oyster) and *Mytilus galloprovincialis* (the Mediterranean mussel), respectively. Other invertebrates for which immune response may be enhanced in the disclosed manner include other mollusks, arthropods such as the crustacean shrimps, prawn and crabs, and cephalopods such as squid, echinoderms such as sea cucumbers and sea urchins. In certain embodiments, disclosed systems and methods may be applied in fish aquaculture, enhancing the immune response of the fish against pathogens such as viruses and bacteria.

[0019] Table 1 provides non limiting examples for invertebrates and vertebrates (fish) and their respective pathogens, which may be handled by disclosed systems and methods applying UV-inactivation of the pathogens to enhance the immune reaction of the respective invertebrates and vertebrates (fish).

Table 1: Organisms (invertebrates and vertebrates) and their respective pathogens, which may be UV-inactivated to enhance immunity of the organisms grown in aquaculture

Host organism in aquaculture	Disease	Pathogen to be UV-inactivated
Abalone (sea snail) (*Haliotis laevigata*, *H. rubra*)	AVG- Abalone Viral Ganglioneuritis	AbHV (Abalone Herpes Virus)
Shrimp/Prawns, e.g., *Litopenaeus vannamei*, *Penaeus monodon* and others	IHHN- Infectious Hypodermal and Haematopoitic Necrosis	IHHNV
Shrimps: *Penaeus monodon* and others	Yellowhead disease	Yellow head virus (YHV)
Penaeid shrimps	Infectious myonecrosis	IMNV (infectious myonecrosis virus)
Shrimp: Macrobrachium rosenbergii	White tail disease	MrNV (*Macrobrachium rosenbergii* nodavirus)
Shrimps: *Litopenaeus vannamei* and others	Taura syndrome	Taura syndrome virus (TSV)
Shrimps: *Litopenaeus vannamei* and others	WSS (White spot syndrome)	WSSV
Squid, mackerel, tuna, sardines, crab, conch, shrimp, and bivalves, such as oysters and clams.	Acute hepatopancreatic necrosis	*Vibrio parahaemolyticus*

[0020] Certain embodiments comprise inactivating specified pathogens using UV radiation, exposing vertebrates such as fish, grown or to be grown in aquaculture, to the specified UV-inactivated pathogens to enhance an immune reaction of the exposed vertebrates (e.g., fish) toward the specified pathogens, and growing the exposed vertebrates (e.g., fish) in aquaculture.

[0021] Certain embodiments comprise an aquaculture system comprising an immunization unit configured to expose vertebrates such as fish to specified UV-inactivated pathogens to enhance an immune reaction of the exposed vertebrates (e.g., fish) toward the specified pathogens, an aquaculture growth unit configured to grow the exposed vertebrates (e.g., fish), and possibly a pathogen-inactivation unit configured to inactivate specified pathogens using UV radiation.

[0022] Certain embodiments comprise immunogenic compositions comprising inactivated pathogens that are listed in **Table 1**, produced by irradiating the respective pathogens with UV radiation.

[0023] Certain embodiments comprise methods of immunologically protecting fish against specified pathogens listed, e.g., in **Table 1**, by irradiating the specified pathogens with UV radiation to yield an immunogenic composition, and exposing the fish to the immunogenic composition to enhance their immune reaction toward the specified pathogens.

[0024] Aquaculture systems and methods are provided, as well as immunogenic compositions and methods of immunologically protecting bivalves against specified pathogens. Methods include inactivating specified pathogens using UV (ultraviolet) radiation, exposing invertebrates grown or to be grown in aquaculture to the specified UV-inactivated pathogens to enhance an immune reaction of the exposed invertebrates toward the specified pathogens, and growing the exposed invertebrates in aquaculture. In a non-limiting example, the methods were demonstrated to increase the immune response of oysters to OsHV-1 following their prior exposure to UV-inactivated OsHV-1.

[0025] Figures 1A, 1D and **1E** are high-level schematic block diagrams of aquaculture systems **100**, according to some embodiments of the invention. **Figures 1B** and **1C** are high-level schematic illustrations of pathogen-inactivation units **101**, according to some embodiments of the invention. In various embodiments, system **100** comprises pathogen-inactivation unit **101** configured to inactivate (stage **210**, see below) specified pathogens **90** using UV radiation **110** and/or an immunization unit **102** configured to expose (stage **220**, see below) invertebrates **80** to specified UV-inactivated pathogens **120** and/or to an immunogenic composition **125** - to enhance an immune reaction of the exposed invertebrates toward the specified pathogens **130**. System **100** may further comprise an aquaculture growth unit **70** configured to grow exposed invertebrates **130** – with higher yields due to their increased immune reaction.

[0026] Figure 1A schematically illustrates UV inactivation by a UV source **114** such as a low pressure and/or medium pressure UV lamp **114** within a lamp enclosure **112** attached to a collimating tube **116** configured to yield collimated radiation **111** that is applied to sample **115**. In certain embodiments, UV radiation may be carried out by one or more UV sources **114** such as low pressure and/or medium pressure UV lamp(s), LEDs (light emitting diodes), or any other UV source. The type of UV source **114** may be selected according to the required wavelengths. UV radiation may comprise radiation within the wavelength range of 200-400nm, and may have peaks e.g., at any of 253.5nm, 265nm, 275nm (e.g., ±5nm, ±10nm, ±15nm, ±20nm, or intermediate values) and e.g., at peak widths of e.g., ±5nm, ±10nm, ±15nm, ±20nm, or intermediate values).

[0027] In certain embodiments, UV inactivation may be carried out on flowing water containing the respective pathogens (see, e.g., **Figure 1B** below), and may involve a variety of optical configurations that are arranged to ensure inactivation of the pathogens. In certain embodiments, a large quantity of UV inactivated pathogens **120** may be prepared in pathogen-inactivation unit **101** (e.g., as an immunogenic composition **125** comprising UV-inactivated pathogens **120** and possibly additives, see example below) and then added gradually or in one or more portions to immunization unit **102** and/or directly to aquaculture growth unit **70** to yield the enhanced immune resistance to specified pathogens **90**.

[0028] It is noted that immunogenic composition **125** may be prepared separately, e.g., in in pathogen-inactivation unit **101** that operates independently of immunization unit **102**. For example, immunogenic composition **125** may be prepared prior to the operation of immunization unit **102**, and be added to it (and/or to aquaculture growth unit **70**) during their operation. Decoupling pathogen-inactivation unit **101** and immunization unit **102** may be advantageous in certain operation schemes, using immunogenic composition **125** (possibly with additives, conservatives etc.) as intermediate material that can be used to generate the immunity in the grown organisms.

[0029] In various embodiments, exposure **220** of invertebrates **80** to UV-inactivated pathogens **120** may be carried out by direct injection and/or by introduction of UV-inactivated pathogens **120** into the water in which invertebrates **80** are held and/or grown. Exposure **220** may be carried out in separate container(s) and/or in aquaculture growth unit **70**, as disclosed below. Exposure **220** of invertebrates **80** to UV-inactivated pathogens **120** may be carried out during larval and/or adult stages of invertebrates **80**. It is noted that the term "water" used herein refers to any water-based liquid used in aquaculture practice, e.g., water with additives. It is emphasized that disclosed UV-inactivation **210** may be configured to maintain whatever pathogen structures are required to initiate the immune response in invertebrates **80**, e.g., cell membranes or other cell structures, specific proteins or other molecular structures, specific genome parts, etc.

[0030] Figures 1B and **1C** illustrate schematically pathogen-inactivation units **101** configured to operate on flowing water, receiving an incoming flow **106** (into which pathogens **90** may be introduced), applying UV radiation **110** to the flow in a conduit **105** - to yield outcoming flow **107**

with UV-inactivated pathogens. It is noted that UV-inactivation **210** may be carried out on static water (as illustrated schematically in **Figure 1A**, possibly using a shutter to define the exposure duration and a stirrer to mix the water) and/or on flowing water (as illustrated schematically in **Figures 1B** and **1C**), according to specific requirements. Applying UV radiation **110** to the flow in conduit **105** may be carried out in various configurations of unit **101**, e.g., as disclosed in any of U.S. Patent Nos. 9,809,467, 10,029,926, 10,294,124 and 10,427,954 incorporated herein by reference in their entirety. For example, UV radiation **110** may be collimated to yield a uniform radiation distribution and/or conduit **105** may be configured to provide internal reflection (e.g., total internal reflection) to uniformly distribute the UV radiation within the water flowing therethrough, ensuring uniform and/or effective UV-activation of the pathogens in flow **106**. In certain embodiments, UV LEDs may be positioned at locations along conduit **105** that provide a uniform UV radiation distribution. In certain embodiments, the UV radiation distribution can be non-uniform but predictable in the sense that a specified threshold of pathogen-inactivation may be ensured.

[0031] The dose and time of exposure may be determined with respect to the volume of the container in static UV irradiation units **101** and/or with respect to the conduit dimensions and flow velocity in dynamic UV irradiation units **101**, in relation to the UV transmission of the water. In certain embodiments, the dimension of the container and duration of retention of the water in static units **101** and/or the conduit dimensions and flow velocity in dynamic UV irradiation units **101** may be selected according to specified throughput and time requirements. For example, when using collimated UV radiation 110, the UV dose may be determined by **Equation 1**:

$$D_{CB} = E_s P_s (1 - R) \frac{L}{d + L} \frac{1 - 10^{-(A_{WL} d)}}{A_{WL} d ln(10)}$$

Equation 1

with D_{CB} denoting the UV dose (mJ/cm^2), Es denoting the average UV intensity (measured before and after irradiating the sample) (mW/cm^2), P_f denoting the Petri factor (unitless), R denoting the reflectance at the air-water interface at 254 nm (unitless), as an example for the applied wavelength, L denoting the distance from the centerline of the lamp (e.g., in embodiments such as illustrated in **Figure 1A**) to the suspension surface (cm), d denoting the depth of the suspension of the pathogens in the water (cm), A_{WL} denoting the UV absorbance at the specific used wavelength (unitless) and t denoting the exposure time (s).

[0032] In certain embodiments, pathogen-inactivation unit **101** may be configured to comprise at least one flow loop **108**, as illustrated schematically in **Figure 1C**, delivering outcoming flow **107** back to conduit **105** and/or to other irradiated conduit(s) as incoming flow **106** to apply UV radiation **110** in a stepwise manner. In certain embodiments, the water may be circulated through same conduit 105 to apply UV radiation **110** multiple times, possibly with intermediate mixing. Using smaller UV doses, the recurring irradiation may provide average uniform pathogen UV-inactivation.

[0033] Figures 1D and **1E** illustrate schematically aquaculture systems **100** in which the immunization of the grown invertebrates is carried out at least partly within aquaculture growth unit **70**. In such embodiments, immunization unit **102** (indicated schematically within aquaculture growth unit **70**) may be a separate compartment within aquaculture unit **70** and/or the immunization may be carried within aquaculture growth unit **70** as a whole.

[0034] In certain embodiments, pathogen-inactivation unit **101** may be adjacent to aquaculture unit **70**, with immunization unit **102** being part of aquaculture unit **70**, as illustrated schematically in **Figure 1D**. For example, pathogens **90** may be introduced to the inlet into aquaculture unit **70** and undergo UV inactivation immediately prior to the entrance of the water into aquaculture unit **70**, so that the enhancing of the immune reaction of the invertebrates to the pathogen may be carried out within aquaculture unit **70** itself. In certain embodiments, illustrated e.g., in **Figure 1E**. Immunogenic composition **125** may be prepared separately and introduced into aquaculture unit **70** (and/or into immunization unit **102** therewithin) on one or more occasions. For example, portions of immunogenic composition **125** may be added to aquaculture unit **70** periodically, possibly corresponding to growth stages of invertebrates grown therewithin.

[0035] Elements from **Figures 1A-1E** may be combined in any operable combination, and the illustration of certain elements in certain figures and not in others merely serves an explanatory purpose and is non-limiting.

[0036] Figure 2 is a high-level flowchart illustrating a method **200**, according to some embodiments of the invention. The method stages may be carried out with respect to system **100** described above, which may optionally be configured to implement method **200**. Method **200** may comprise the following stages, irrespective of their order.

[0037] Method **200** may comprise inactivating specified pathogens using UV radiation (stage **210**), exposing invertebrates grown or to be grown in aquaculture to the specified UV-inactivated pathogens (stage **220**) to enhance an immune reaction of the exposed invertebrates toward the specified pathogens, and growing the exposed invertebrates in aquaculture (stage **230**). For example, method **200** may be used as method **200** of immunologically protecting bivalves against specified pathogens, comprising: irradiating the specified pathogens with UV radiation to yield an immunogenic composition (stage **212**), and exposing the bivalves to the immunogenic composition to enhance their immune reaction toward the specified pathogens (stage **222**), followed by growing the exposed bivalves in aquaculture (stage **230**).

[0038] In various embodiments, inactivation **210** of the specified pathogens may be carried out in a static setting using collimated UV radiation (stage **214**) and/or inactivation **210** of the specified pathogens may be carried out in a dynamic setting, applying UV radiation to a flow carrying the pathogens (stage **216**) – for example by delivering uniform UV radiation to a conduit supporting the flow. In certain embodiments, applying UV radiation to a flow carrying the pathogens may be carried out by delivering UV radiation repeatedly to one or more conduits supporting the flow and comprising at least one flow loop.

[0039] In various embodiments, exposing **220** of the invertebrates to the specified UV-inactivated pathogens may be carried out by injecting an immunogenic composition comprising the specified UV-inactivated pathogens to the invertebrates (stage **224**) and/or by adding the immunogenic composition into water in which the invertebrates are held and/or grown (stage **226**), possibly repeatedly (stage **228**) to maintain a required immunity level and/or to parallel developmental stage of the invertebrates, providing immunity to consecutive generations and/or developmental stages.

[0040] Certain embodiments comprise immunogenic composition **125** comprising inactivated OsHV-1 (ostreid herpesvirus 1) and/or inactivated *Vibrio* bacteria, produced by irradiating **212** the OsHV-1 viruses and/or the *Vibrio* bacteria with UV radiation **110**.

[0041] The following experimental data illustrates the efficiency of disclosed systems, methods and composition in enhancing the immune response of *Crassostrea gigas* (the Pacific oyster) to OsHV-1, using UV-inactivated pathogens.

[0042] **Figures 3A-3C** illustrate the setting and results of experiments conducted to show the enhancing the immune response of *C. gigas* to OsHV-1 using UV-inactivated pathogens, according to some embodiments of the invention. **Figure 3A** illustrate schematically the experimental setting and **Figures 3B** and **3C** provide corresponding experimental results.

[0043] As illustrated schematically in **Figure 3A**, four sets of virus treatments were checked on the oysters that were grown without and with exposure to the active viruses. Specifically, the four treatments included (i) UV-treated OsHV-1, (ii) UV-treated non-viral suspension as a negative control (denoted "no virus, UV"), (iii) filtered seawater as a negative control (denoted "FSW") and (iv) Poly (I:C), indicating virus-mimic synthetic double stranded RNA (dsRNA) which is known to be a key signature of viral infection and widely used as a viral mimic in vertebrates - as a positive control (denoted "PIC"). The UV treatment in (i) and (ii) were carried out for **105** seconds using low pressure UV as illustrated schematically in **Figure 1A**. UV inactivation was carried out by a low pressure UV mercury arc lamp **114** within lamp enclosure **112** attached to collimating tube **116** configured to yield collimated radiation **111** (e.g., at 254nm wavelength) – that is applied to sample **115**, placed on a magnetic stirrer **117**. It is noted that this experimental setting is not limiting, and that various system configuration may be used, as illustrated, e.g., in any combination of features from **Figures 1A-1E** discussed above.

[0044] Each of the treatments was applied by injection to n=140 oysters, which were then split into two groups, with 70 oysters each. One group was exposed to filtered seawater (four treatments + SW) and the other group was exposed to OsHV-1 contaminated seawater (four treatments + CSW) – with the number of oysters in each sub-group shown in **Figure 3A**. In each group of 70 oysters, 40 oysters were used for sampling and 30 oysters were used for mortality monitoring. OsHV-1 contaminated seawater was prepared by infecting healthy oysters with **100**µL of OsHV-1 and using the water they were in after 24h as CSW. All sub-groups were held under similar conditions and were sampled every day for 8 days. **Figure 3B** illustrates the survival rates of the eight subgroups during the experiment, in the CSW group, and **Figure 3C** illustrated the viral load of oysters in the sub-groups at T2, denoting samples taken 24 hours after the exposure to SW/CSW and at T3, denoting samples taken 48 hours after the exposure to SW/CSW, in the CSW group.

[0045] As seen in **Figure 3B**, while the negative controls (treatments (ii) and (iii)) resulted in declining oyster populations in contaminated seawater (CSW), reaching 23% and 13% survival 7 days post-infection, respectively - UV-treated OsHV-1 (treatment (i)) demonstrated significant (****$p<0.0001$) higher survival rates than the negative controls (FSW-CSW), with 96.7% survival 7 days post-infection, and similar results to treatment (iv) with poly(I:C), with 100% survival 7 days post-infection, no significant differences between treatments (i) and (iv).

[0046] As seen in **Figure 3C**, while the negative controls (treatments (ii) and (iii)) reached high viral loads 48 hours after infection in CSW ($4.8 \cdot 10^5$ and $1.8 \cdot 10^6$ genome units/extract DNA, respectively) - UV-treated OsHV-1 (treatment (i)) demonstrated significantly lower viral loads (p-value<0.05, mean load $5.6 \cdot 10^4$ genome unit/extract DNA) which were similar to treatment (iv) with poly(I:C) with a mean viral load of $1.73 \cdot 10^2$, with no significant differences between treatments (i) and (iv) 48 hours after infection in CSW.

[0047] It has been further found out that disclosed methods yield activation of antiviral immune genes in *C. gigas*, as illustrated in **Table 2** and **Figure 4**. **Table 2** provides the relative expression levels for eight candidate immune/cell death genes under treatment conditions (i) through (iv) – 24 hours after treatment with respect to naive oysters. Candidate genes were chosen according to the literature, as belonging to (a) the interferons and NF-κB pathways: RLR (RIG-I-like receptor gene, MyD88-1 (Myeloid differentiation factor 88-1, which is a TLR adaptor protein,), IRF-2 (interferon regulation factor 2, transcription factor); (b) antiviral effectors-:ADAR (adenosine deaminase, RNA specific, antiviral effector), viperin (antiviral effector), IAP (inhibitor of apoptosis proteins) and (c) the autophagy pathway: ATG8 (Autophagy-related protein 8), Beclin-1 (autophagy regulation). The results show that UV-inactivated virus exposure induced an upregulation of antiviral genes (except for ADAR) and autophagy-related genes, similar but to a lesser extent than for the positive control treatment (iv) of exposure to poly(I:C).

Table 2 - relative expression levels of eight immune genes to the four treatments

Treatment	Immune genes							
	Viperin	RLR	ADAR	IRF2	MyD88-1	ATG8	Beclin	IAP18
(i) Virus, UV	9.3	3.1	0.3	5.1	16.8	0.1	0.2	0.6
(ii) Only UV	1.9	0.7	0.3	0.8	13.5	0.2	0.2	0.4
(iii) FSW	0.9	0.8	0.3	0.6	11.6	0.2	0.2	0.3
(vi) Poly (I:C)	28.6	4.5	0.9	23.9	59.2	0.4	0.3	4.8
Values above 1 indicate up-regulation of the gene 24h after exposure, values between 0.5-1 indicate stable expression of the respective gene and values under 0.5 indicate down-regulation of the respective gene.								

[0048] Figure 4 illustrates the effect of treatments (i) to (iv) on the expression of each of the eight genes presented in **Table 2**. **Figure 4** provides violin plots of the expression patterns for the eight candidate genes, under treatments (i) through (iv). Significance of the differences between the treatments was evaluated using Dunn's Multiple Comparison Test; ns > 0.05, **p<0.01, ***p<0.001, ****p<0.0001. This more detailed presentation of the gene activation results corroborates the disclosed action mechanism of exposure to UV-inactivated viruses.

[0049] To conclude, the results demonstrate that disclosed exposure of invertebrates to UV-inactivated pathogens enhance their immune response toward these pathogens and improve aquaculture practice.

[0050] In the above description, an embodiment is an example or implementation of the invention. The various appearances of "one embodiment", "an embodiment", "certain embodiments" or "some embodiments" do not necessarily all refer to the same embodiments. Although various features of the invention may be described in the context of a single embodiment, the features may also be provided separately or in any suitable combination. Conversely, although the invention may be described herein in the context of separate embodiments for clarity, the invention may also be implemented in a single embodiment. Certain embodiments of the invention may include features from different embodiments disclosed above, and certain embodiments may incorporate elements from other embodiments disclosed above. The disclosure of elements of the invention in the context of a specific embodiment is not to be taken as limiting their use in the specific embodiment alone. Furthermore, it is to be understood that the invention can be carried out or practiced in various ways and that the invention can be implemented in certain embodiments other than the ones outlined in the description above.

[0051] The invention is not limited to those diagrams or to the corresponding descriptions. For example, flow need not move through each illustrated box or state, or in exactly the same order as illustrated and described. Meanings of technical and scientific terms used herein are to be commonly understood as by one of ordinary skill in the art to which the invention belongs, unless otherwise defined. While the invention has been described with respect to a limited number of embodiments, these should not be construed as limitations on the scope of the invention, but rather as exemplifications of some of the preferred embodiments. Other possible variations, modifications, and applications are also within the scope of the invention. Accordingly, the scope of the invention should not be limited by what has thus far been described, but by the appended claims and their legal equivalents.

CLAIMS

What is claimed is:

1. A method comprising:

   inactivating specified pathogens using UV (ultraviolet) radiation,

   exposing invertebrates grown or to be grown in aquaculture to the specified UV-inactivated pathogens to enhance an immune reaction of the exposed invertebrates toward the specified pathogens, and

   growing the exposed invertebrates in aquaculture.

2. The method of claim 1, wherein the exposing of the invertebrates to the specified UV-inactivated pathogens is carried out by injecting an immunogenic composition comprising the specified UV-inactivated pathogens to the invertebrates.

3. The method of claim 1 or 2, wherein the exposing of the invertebrates to the specified UV-inactivated pathogens is carried out by adding the immunogenic composition into water in which the invertebrates are held and/or grown.

4. The method of claim 3, wherein the adding of the immunogenic composition is carried out repeatedly.

5. The method of any one of claims 1-4, wherein the inactivation of the specified pathogens is carried out in a static setting using collimated UV radiation.

6. The method of any one of claims 1-4, wherein the inactivation of the specified pathogens is carried out in a dynamic setting, applying UV radiation to a flow carrying the pathogens.

7. The method of claim 6, wherein the inactivation of the specified pathogens is carried out by delivering uniform UV radiation to a conduit supporting the flow.

8. The method of claim 6, wherein the inactivation of the specified pathogens is carried out by delivering UV radiation repeatedly to one or more conduits supporting the flow and comprising at least one flow loop.

9. The method of any one of claims 1-8,

   wherein the invertebrates comprise bivalves and the method comprises immunologically protecting the bivalves against the specified pathogens,

   wherein the inactivating comprises irradiating the specified pathogens with UV radiation to yield an immunogenic composition, and

   wherein the growing comprises exposing the bivalves to the immunogenic composition to enhance their immune reaction toward the specified pathogens.

10. An aquaculture system comprising:

an immunization unit configured to expose invertebrates to specified UV-inactivated pathogens to enhance an immune reaction of the exposed invertebrates toward the specified pathogens, and

an aquaculture growth unit configured to grow the exposed invertebrates.

11. The aquaculture system of claim 10, wherein the immunization unit is part of and/or within the aquaculture growth unit.

12. The aquaculture system of claim 10 or 11, wherein the immunization unit is configured to expose the invertebrates to the specified UV-inactivated pathogens by injecting an immunogenic composition comprising the specified UV-inactivated pathogens to the invertebrates and/or by adding the immunogenic composition into water in which the invertebrates are held.

13. The aquaculture system of any one of claims 10-12, further comprising a pathogen-inactivation unit configured to inactivate specified pathogens using UV radiation.

14. The aquaculture system of claim 13, wherein the pathogen-inactivation unit is configured to inactivate the specified pathogens in a static setting using collimated UV radiation.

15. The aquaculture system of claim 13, wherein the pathogen-inactivation unit is configured to inactivate the specified pathogens in a dynamic setting, applying UV radiation to a flow carrying the pathogens.

16. The aquaculture system of claim 15, wherein the pathogen-inactivation unit is configured to deliver uniform UV radiation to a conduit supporting the flow.

17. The aquaculture system of claim 15, wherein the pathogen-inactivation unit is configured to deliver UV radiation repeatedly to one or more conduits supporting the flow.

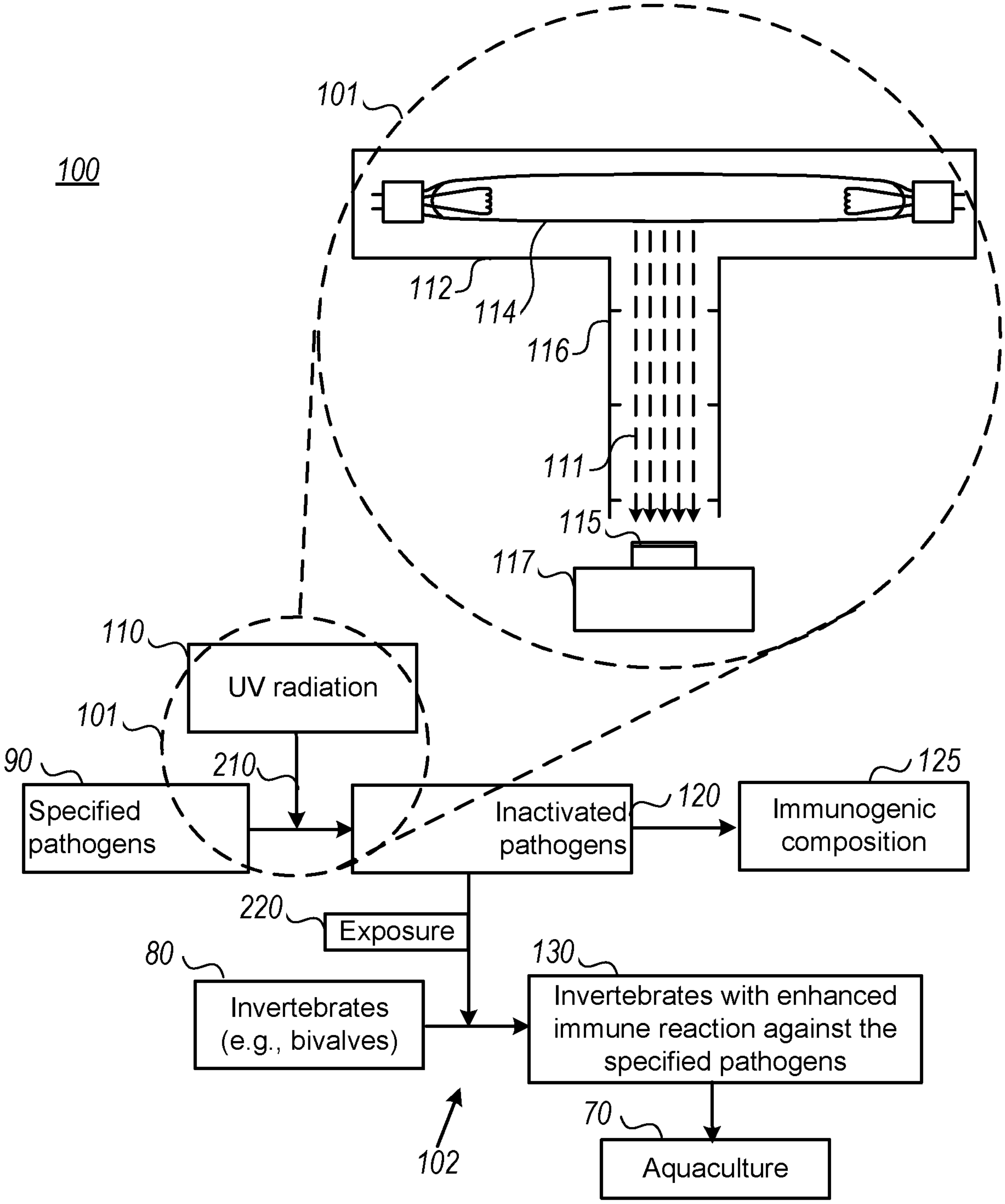
18. The aquaculture system of any one of claims 15-17, wherein the dynamic setting comprises at least one flow loop onto which the UV radiation is applied.

19. An immunogenic composition comprising inactivated OsHV-1 (ostreid herpesvirus 1) and/or inactivated *Vibrio* bacteria, produced by irradiating the OsHV-1 viruses and/or the *Vibrio* bacteria with UV radiation.

20. A method of immunologically protecting bivalves against specified pathogens, the method comprising:

irradiating the specified pathogens with UV radiation to yield an immunogenic composition, and exposing the bivalves to the immunogenic composition to enhance their immune reaction toward the specified pathogens.

21. The method of claim 20, wherein the exposing of the bivalves is carried out by injecting the immunogenic composition comprising the specified UV-inactivated pathogens to the bivalves.
22. The method of claim 20 or 21, wherein the exposing of the bivalves is carried out by adding the immunogenic composition into water in which the bivalves are held and/or grown.
23. The method of claim 22, wherein the adding of the immunogenic composition is carried out repeatedly.
24. The method of any one of claims 20-23, wherein the irradiating the specified pathogens is carried out in a static setting using collimated UV radiation.
25. The method of any one of claims 20-23, wherein the irradiating the specified pathogens is carried out in a dynamic setting, applying UV radiation to a flow carrying the pathogens.
26. The method of claim 25, wherein the irradiating is carried out by delivering uniform UV radiation to a conduit supporting the flow.
27. The method of claim 25, wherein the irradiating is carried out by delivering UV radiation repeatedly to one or more conduits supporting the flow and comprising at least one flow loop.



100
101
112
114
116
111
115
117
110
UV radiation
101
90
210
Specified pathogens
Inactivated pathogens
120
125
Immunogenic composition
220
Exposure
80
Invertebrates (e.g., bivalves)
130
Invertebrates with enhanced immune reaction against the specified pathogens
70
Aquaculture
102

Figure 1A

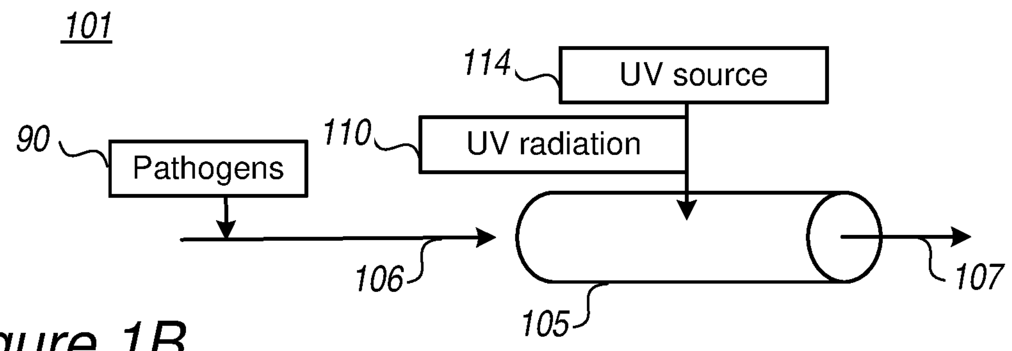


Figure 1B

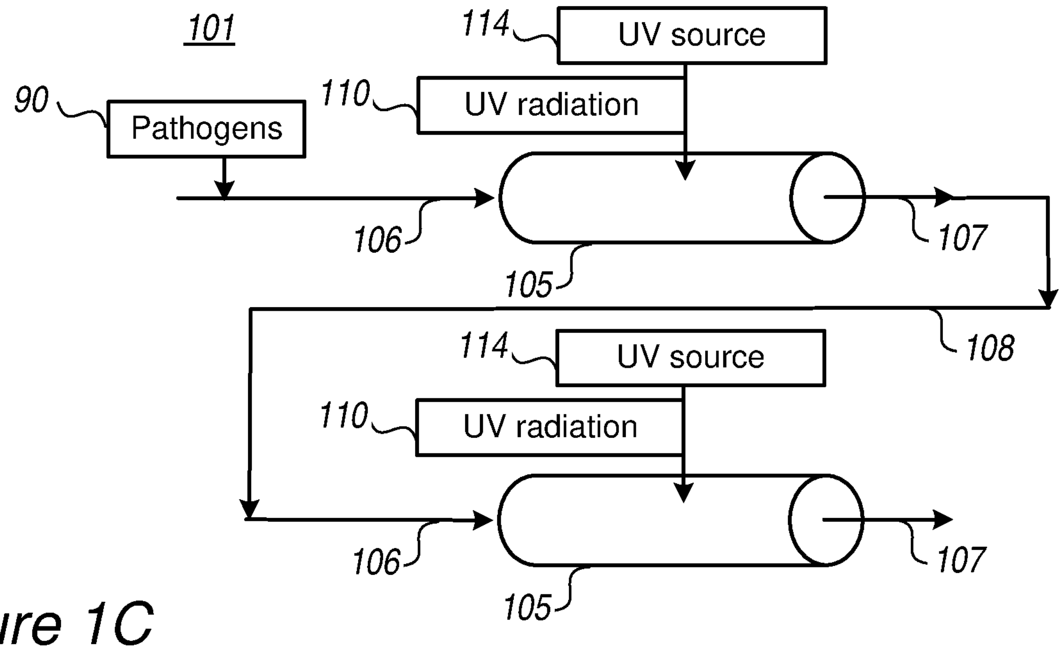


Figure 1C

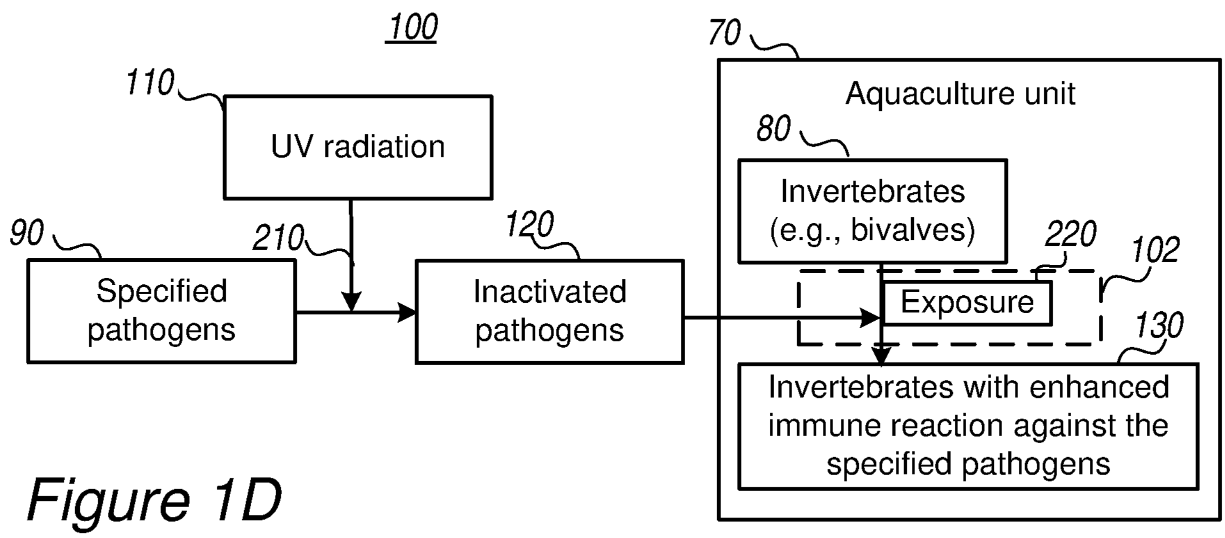


Figure 1D

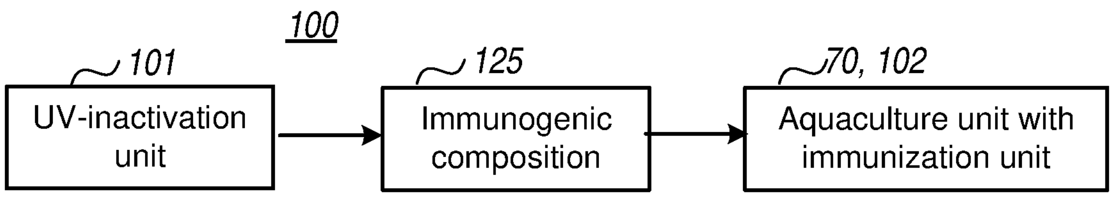


Figure 1E

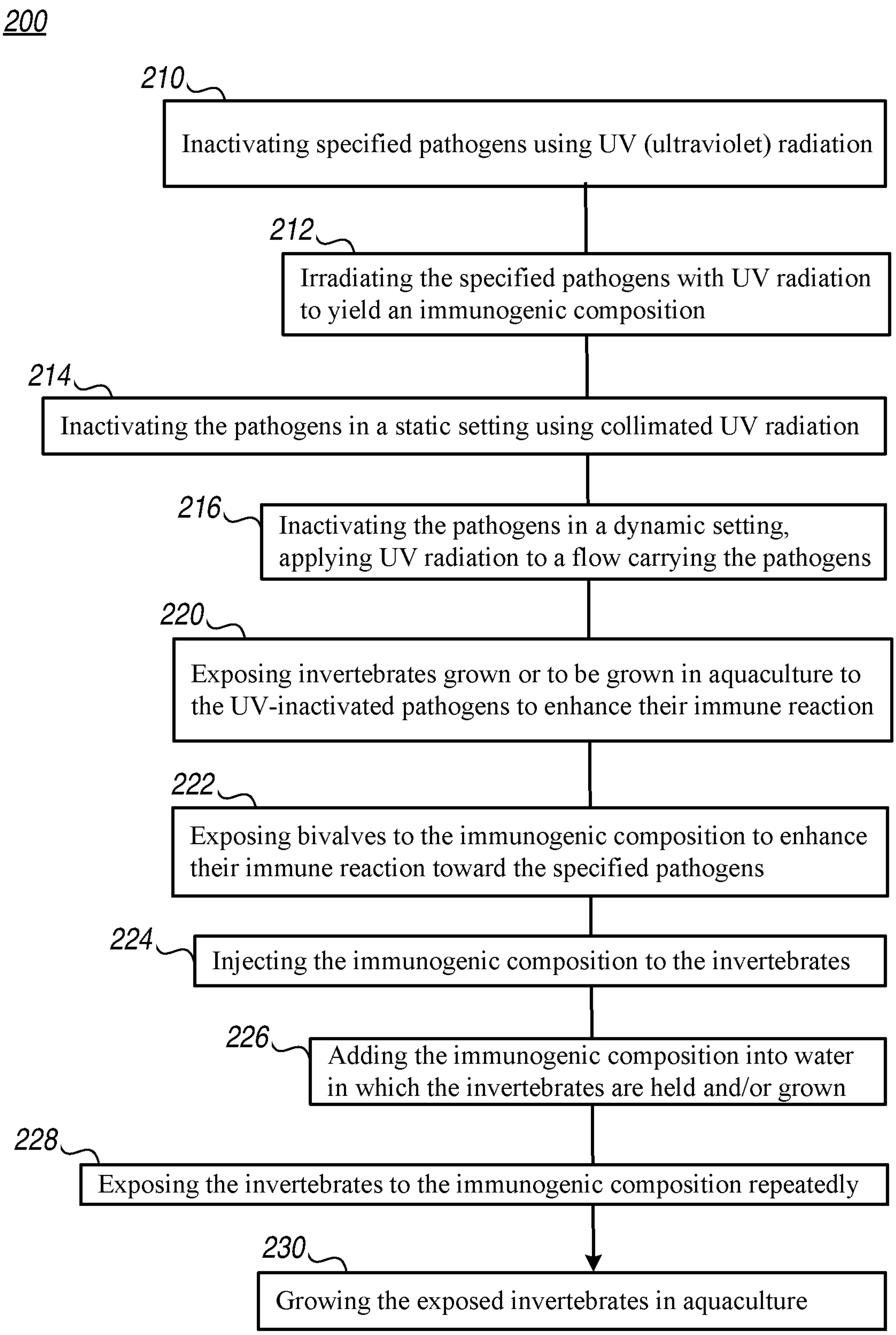
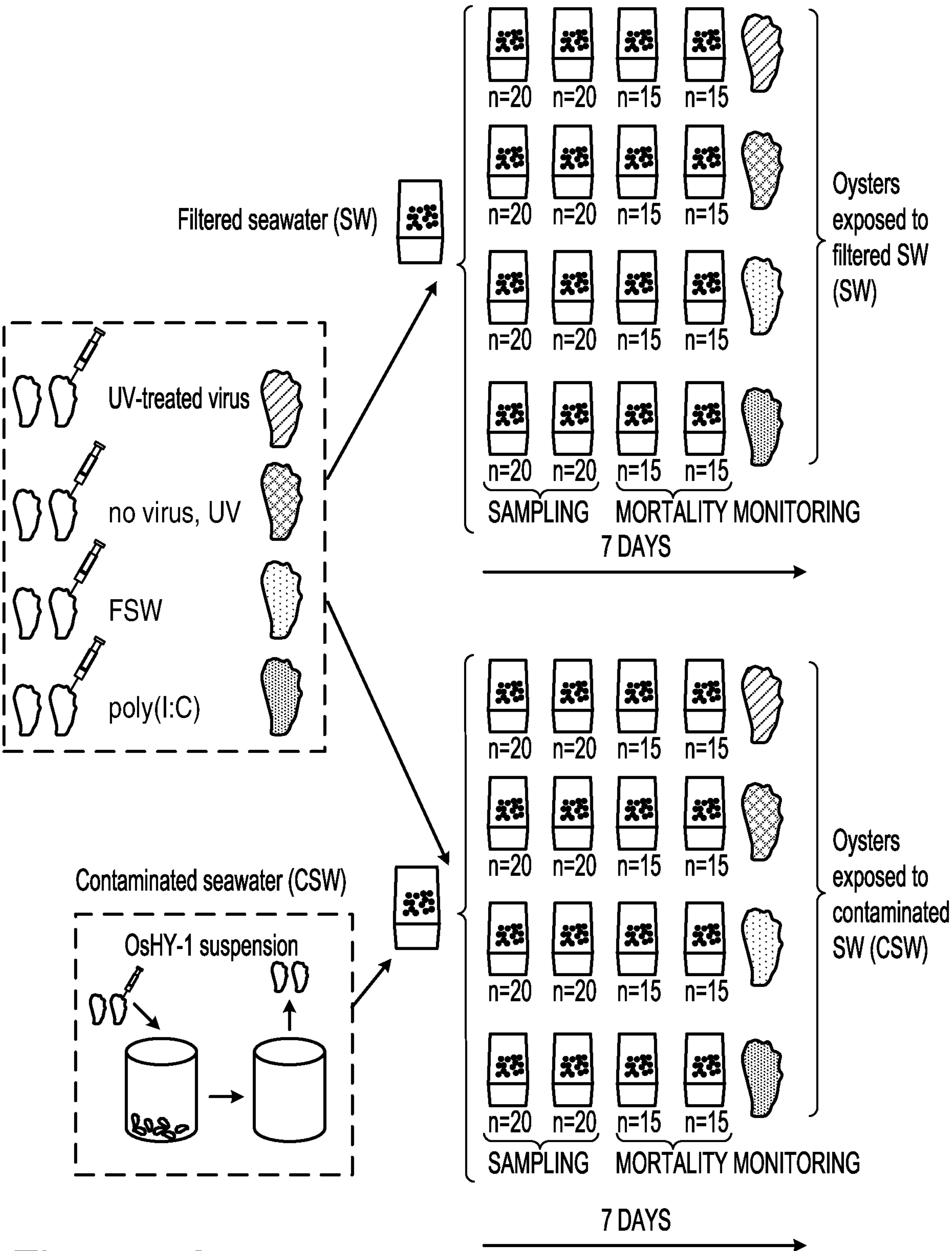

200
210
Inactivating specified pathogens using UV (ultraviolet) radiation
212
Irradiating the specified pathogens with UV radiation to yield an immunogenic composition
214
Inactivating the pathogens in a static setting using collimated UV radiation
216
Inactivating the pathogens in a dynamic setting, applying UV radiation to a flow carrying the pathogens
220
Exposing invertebrates grown or to be grown in aquaculture to the UV-inactivated pathogens to enhance their immune reaction
222
Exposing bivalves to the immunogenic composition to enhance their immune reaction toward the specified pathogens
224
Injecting the immunogenic composition to the invertebrates
226
Adding the immunogenic composition into water in which the invertebrates are held and/or grown
228
Exposing the invertebrates to the immunogenic composition repeatedly
230
Growing the exposed invertebrates in aquaculture

Figure 2



Filtered seawater (SW)
UV-treated virus
no virus, UV
FSW
poly(I:C)
n=20 n=20 n=15 n=15
n=20 n=20 n=15 n=15
n=20 n=20 n=15 n=15
n=20 n=20 n=15 n=15
SAMPLING
MORTALITY MONITORING
7 DAYS
Oysters exposed to filtered SW (SW)
Contaminated seawater (CSW)
OsHV-1 suspension
n=20 n=20 n=15 n=15
n=20 n=20 n=15 n=15
n=20 n=20 n=15 n=15
n=20 n=20 n=15 n=15
SAMPLING
MORTALITY MONITORING
7 DAYS
Oysters exposed to contaminated SW (CSW)

Figure 3A

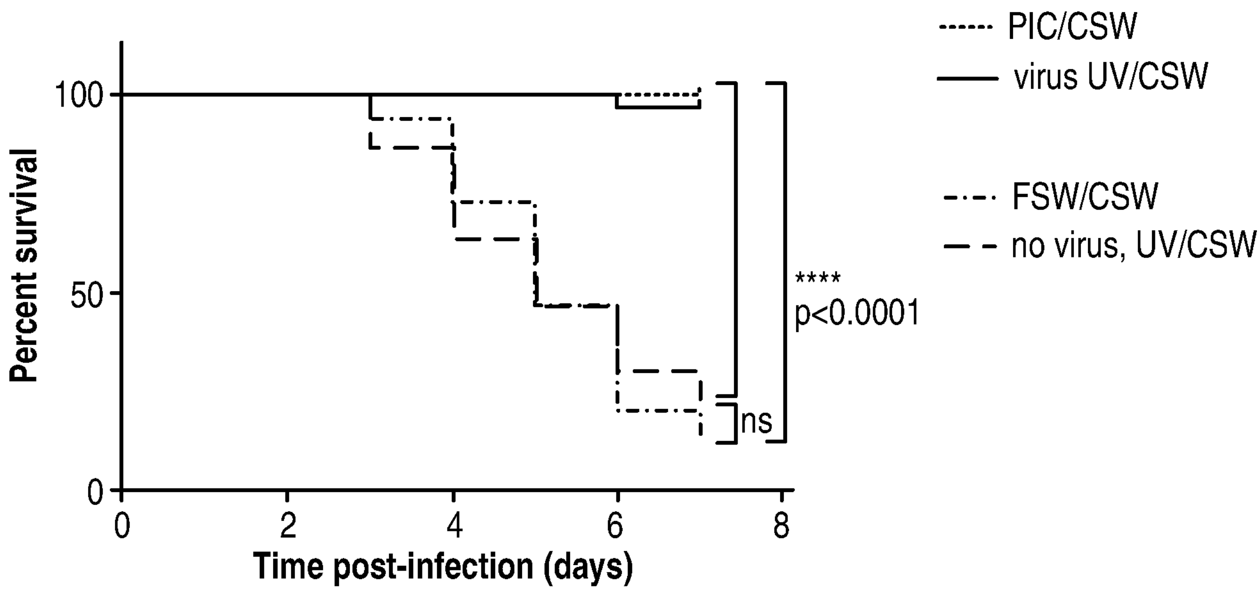


Figure 3B

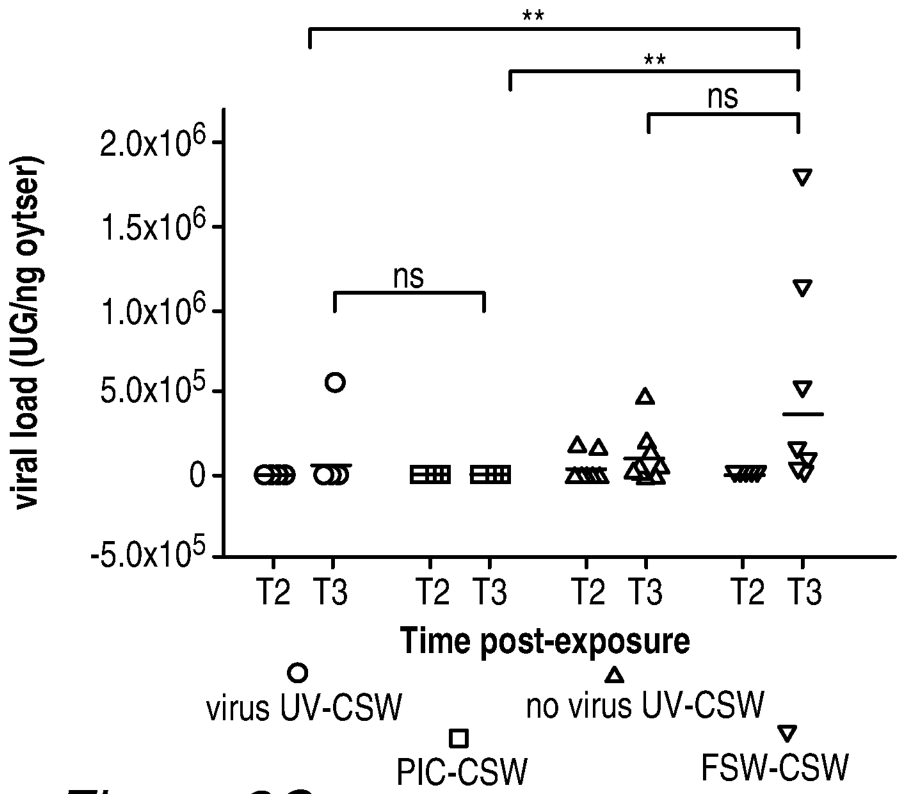


Figure 3C

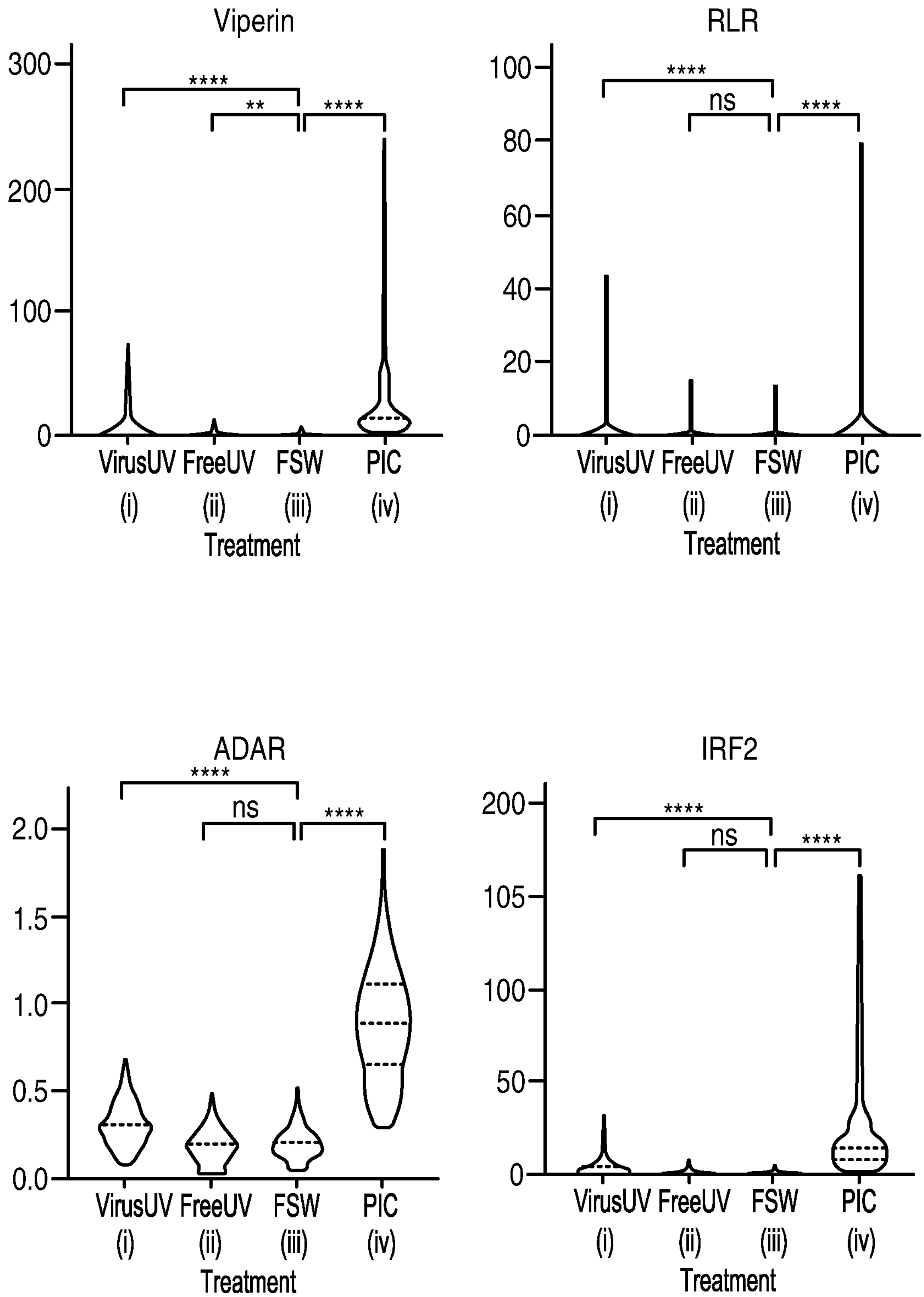

Viperin
300
200
100
0
****
**
****
VirusUV FreeUV FSW PIC
(i) (ii) (iii) (iv)
Treatment
RLR
100
80
60
40
20
0
****
ns
****
VirusUV FreeUV FSW PIC
(i) (ii) (iii) (iv)
Treatment
ADAR
2.0
1.5
1.0
0.5
0.0
****
ns
****
VirusUV FreeUV FSW PIC
(i) (ii) (iii) (iv)
Treatment
IRF2
200
105
100
50
0
****
ns
****
VirusUV FreeUV FSW PIC
(i) (ii) (iii) (iv)
Treatment

Figure 4

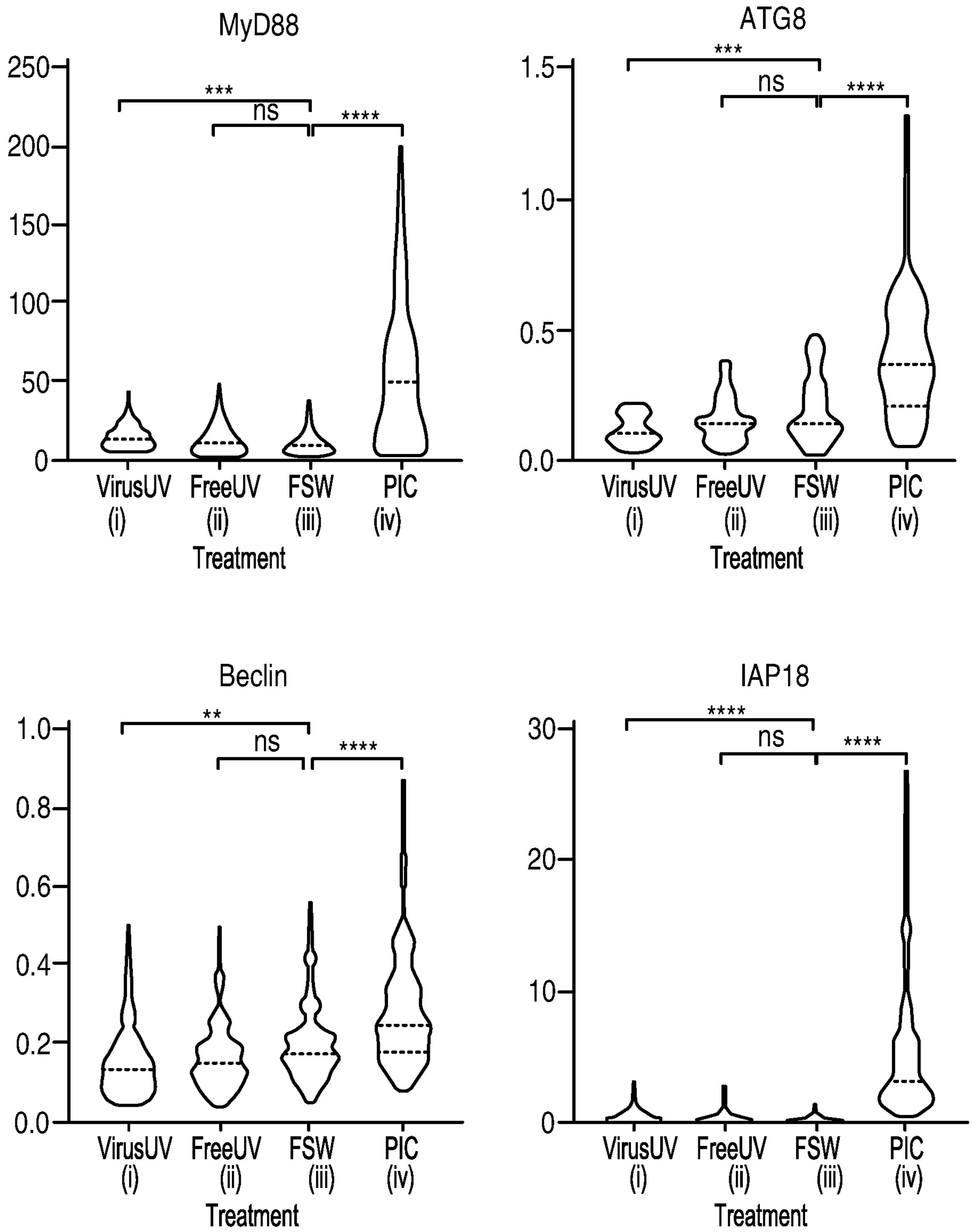


Figure 4 (continued)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IL2021/050176

A. CLASSIFICATION OF SUBJECT MATTER
IPC (20210101) A01K 61/54, A61L 2/00, A61L 2/10, C12N 1/20
CPC (20190801) A01K 61/54, A61L 2/0047, A61L 2/10, C12N 1/20
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC (20210101) A01K 61/54, A61L 2/00, A61L 2/10
CPC (20190801) A01K 61/54, A61L 2/0047, A61L 2/10

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Databases consulted: PATENTSCOPE, THOMSON INNOVATION, Esp@cenet, Google Patents, CAPLUS, BIOSIS, Google Scholar, Derwent Innovation
Search terms used: ultraviolet pathogen aquaculture invertebrates immune

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2018044204 A1 Litchi et al 15 Feb 2018 (2018/02/15) abstract, paragraphs 27-29, 32-33, fig. 1 and fig. 3	1-8,10-19
Y	Itami, T., Y. Takahashi, and Y. Nakamura. "Efficacy of vaccination against vibriosis in cultured kuruma prawns Penaeus japonicus." Journal of Aquatic Animal Health 1.3 (1989): 238-242. Itami et al 01 Sep 1989 (1989/09/01) abstract, page 238 right column lines 7-21	1-8,10-19

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 May 2021	09 Jun 2021

Name and mailing address of the ISA:	Authorized officer
Israel Patent Office Technology Park, Bldg.5, Malcha, Jerusalem, 9695101, Israel Email address: pctoffice@justice.gov.il	POUNY Yehonathan Telephone No. 972-73-3927124

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/IL2021/050176

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		EP 2969966 B1	02 May 2018
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		US 9809467 B2	07 Nov 2017
		WO 2014141269 A1	18 Sep 2014