



## Detection of non-phytoplankton-eating zooplankton within a volume of water

Nielsen, Josefine Holm; Sørensen, Mikkel Brydegaard; Prangma, Jord Cornelis; Rodrigo, Peter John

*Publication date:*  
2020

*Document Version*  
Publisher's PDF, also known as Version of record

[Link back to DTU Orbit](#)

*Citation (APA):*  
Nielsen, J. H., Sørensen, M. B., Prangma, J. C., & Rodrigo, P. J. (2020). Detection of non-phytoplankton-eating zooplankton within a volume of water. (Patent No. WO2020239833).

---

### General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.



(51) International Patent Classification:

G01N 21/64 (2006.01)

(21) International Application Number:

PCT/EP2020/064696

(22) International Filing Date:

27 May 2020 (27.05.2020)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

19177357.1 29 May 2019 (29.05.2019) EP  
20165650.1 25 March 2020 (25.03.2020) EP

(71) Applicant: FAUNAPHOTONICS AGRICULTURE & ENVIRONMENTAL A/S [DK/DK]; Stoberigade 12, 2450 Copenhagen K. (DK).

(72) Inventors: NIELSEN, Josefine Holm; Ryetvej 31, 1. th, 3500 Værløse (DK). SORENSEN, Mikkel Brydegaard; Pistolvagen 7, 226 49 Lund (SE). PRANGSMA, Jord Cornelis; Jonker Sloetlaan 22, 6721 VP Bennekom (NL). RODRIGO, Peter John; Markmandsgade 16, 2. tv., 2300 København S (DK).

(74) Agent: GUARDIAN IP CONSULTING I/S; Diplomvej, Building 381, 2800 Kgs. Lyngby (DK).

(81) Designated States (unless otherwise indicated, for every kind of national protection available):

AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available):

ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— with international search report (Art. 21(3))

(54) Title: DETECTION OF NON-PHYTOPLANKTON-EATING ZOOPLANKTON WITHIN A VOLUME OF WATER

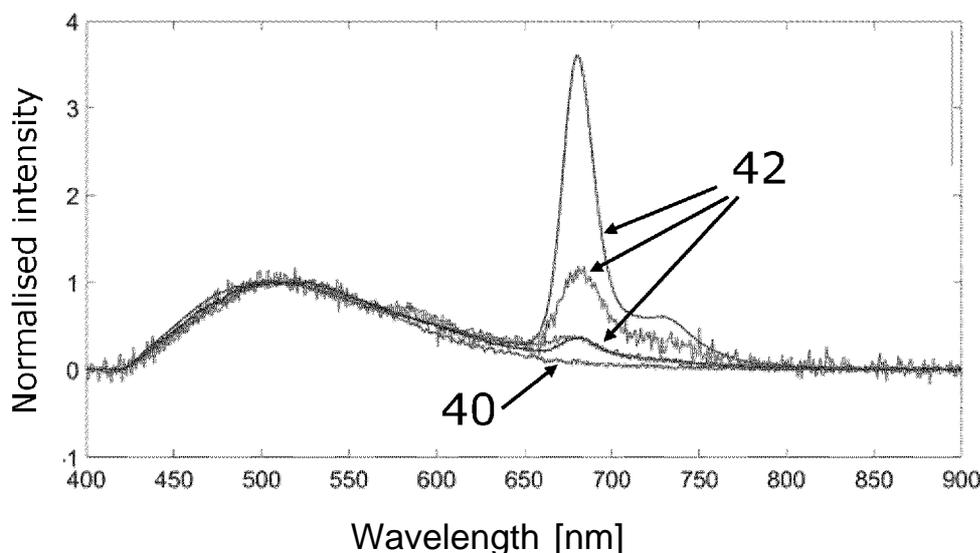


FIG. 4

(57) Abstract: A method and an apparatus for detecting ectoparasites such as salmon lice within a volume of water. Zooplankton are detected within a volume of water and fluorescence from chlorophyll is used to distinguish non-phytoplankton-eating zooplankton (40), such as salmon lice, from phytoplankton-eating zooplankton (42).



DETECTION OF NON-PHYTOPLANKTON-EATING ZOOPLANKTON WITHIN A  
VOLUME OF WATER

TECHNICAL FIELD

- 5 The present disclosure relates to the detection of ectoparasites such as salmon lice within a volume of water.

BACKGROUND

Salmon lice, (*Salmonis*), are ectoparasites, which host primarily on salmon and  
10 have a significant economic impact on the salmon farming industry due to salmon lice control, treatment and reduced health and growth of the infected fish. In addition, infestations of salmon lice in farms may spread and affect populations of wild salmon and trout thus additionally impacting wildlife.

- 15 Treatment and monitoring today is primarily targeted towards the parasitic life stages of the lice, i.e. the stages during which the louse is attached to a host.

In EP 2 962 556 monitoring of ectoparasites on fish is done by illuminating a fish with illumination radiation capable of inducing fluorescence in ectoparasites and  
20 detecting any induced fluorescence using one or more detectors. Any ectoparasites detected using the method and system of EP 2 962 556 are ones that have attached themselves to the fish and are in their parasitic life stage.

More knowledge about the abundance and distribution of the planktonic, larval  
25 stages in which the lice are freely swimming could improve preventive treatment methods, treatment planning and mathematical life cycle and spread models. However, such knowledge is currently limited to what can be gained from manual sampling using plankton nets or water pumps, which is costly, time-consuming and susceptible to local variations. As such a method for real-time, automated  
30 detection of larval salmon lice is a desired alternative.

It is generally desirable to provide a method and an apparatus for detecting freely swimming non-phytoplankton-eating zooplankton within a volume of water.

Plankton, which are organisms living in bodies of water, are divided into zooplankton (animals) and phytoplankton (plants). A characteristic of plankton is its inability to swim against a current and while some are able to change direction or rise and sink, they primarily drift in water. Freely swimming is therefore to be understood in the sense that the zooplankton is not attached to anything, e.g. a host.

#### SUMMARY

Various methods may be employed to detect zooplankton within a volume of water. When zooplankton within the volume of water is detected, various aspects disclosed herein utilize the autofluorescence of chlorophyll to distinguish between phytoplankton-eating and non-phytoplankton-eating zooplankton, i.e. between zooplankton, which eat plants and zooplankton, which do not.

In the larval stage, ectoparasites such as salmon lice do not eat and therefore they may be distinguished from similar zooplankton that feed on phytoplankton, by examining whether undigested chlorophyll is present within the detected zooplankton. Thus, the fluorescence from chlorophyll is a relevant signature that may be exploited in monitoring, possibly real-time, and classification of ectoparasites such as e.g. salmon lice versus phytoplankton-eating zooplankton.

In an aspect, a method for detecting freely swimming non-phytoplankton-eating zooplankton is disclosed. The method comprises:

- illuminating a volume of water with a first illumination radiation comprising a first illumination wavelength, the first illumination radiation being capable of inducing autofluorescence of chlorophyll;
- detecting radiation received from the volume of water;
- determining whether zooplankton is present within the volume of water based on the detected radiation;
- measuring an amount of autofluorescence from chlorophyll from a zooplankton present within the volume of water;
- identifying whether the zooplankton determined to be present within the volume of water is a non-phytoplankton-eating zooplankton based on the measured amount of autofluorescence from chlorophyll.

The different acts of the method are not to be construed as being performed in a set order and the acts as given in the method may be performed in a different order to that in which they are listed. For example, the detecting and/or determining acts may be performed before, concurrently with or subsequent to  
5 the illumination with the first illumination radiation.

When measuring an amount of autofluorescence from chlorophyll from a zooplankton present within the volume of water, the fluorescent light will typically originate from undigested chlorophyll within the zooplankton. The characteristics  
10 of radiation capable of inducing autofluorescence of chlorophyll is well known.

In some embodiments, detecting radiation received from the volume of water comprises capturing an image of the volume of water and determining whether zooplankton is present within the volume of water using a particle finding  
15 algorithm, such as e.g. a particle detection algorithm, an image segmentation algorithm or an event identification and extraction algorithm, to locate zooplankton within the captured image. For example, to this end, a processing unit may process a captured image and/or other measurement results so as to detect one or more features and/or other indicators of the presence of one or  
20 more organisms in the volume of water, e.g. within a predetermined time period, a sliding window or the like. The processing unit may identify individual organisms and/or determine an estimate of the population of organisms in the volume of water.

25 A captured image may be any spatially resolved distribution of light intensities, e.g. captured by an array of light sensitive elements and/or by performing a scanning across a volume of water. In particular detecting radiation may include capturing a one-dimensional image, a 2-dimensional image and/or a three-dimensional image. For example, a one-dimensional image may be obtained by a  
30 sensor having a one-dimensional array of light sensitive elements, e.g. a CCD camera with a sensor that only has one row of pixels. Similarly, a two-dimensional image may be obtained by a sensor having a two-dimensional array of light sensitive elements. Examples of two-dimensional arrays include a quadrant detector and detector arrays, e.g. CCD cameras, having a higher spatial  
35 resolution. Generally, the light sensitive elements of an array of light sensitive

elements may be configured, e.g. by means of one or more lenses and/or other optical elements, to receive light from respective parts of the volume of water.

In some embodiments, measuring an amount of autofluorescence from chlorophyll  
5 from a zooplankton present within the volume of water comprises measuring an intensity of fluorescent light from chlorophyll, the fluorescent light being received from the volume of water. In particular, the fluorescent light may be fluorescent light from zooplankton within the body of water responsive to being illuminated with the first illumination radiation.

10

Generally, the determination of the presence of zooplankton in the body of water and the measurement of autofluorescence from chlorophyll are based on radiation originating from the zooplankton and the chlorophyll itself. Embodiments of the method and system described herein do not require use of any dyes or other  
15 detectable substances to be distributed in the body of water. Instead the detection of the presence of the zooplankton is based on fluorescent light emitted by the zooplankton itself (also referred to as innate fluorescence) and/or based on light reflected by the zooplankton itself. Similarly, the method is based on a measurement of the autofluorescence by chlorophyll itself, in particular by the  
20 chlorophyll present in the gut of the zooplankton.

Generally, an intensity of light may e.g. be measured as an intensity as a function of wavelength or as an intensity from within a wavelength band, e.g. an accumulated intensity or counts.

25

In some embodiments, the measured autofluorescence from chlorophyll is recorded in a wavelength range having end points within the range 650 - 750 nm, preferably within the range 650 - 725 nm, most preferably within the range 650 - 700 nm.

30

In some embodiments the detected radiation, based on which the process determines whether zooplankton is present within the volume of water, is detected responsive to illumination of the volume of water with the first illumination radiation. Alternatively, the detected radiation may be detected  
35 responsive to illumination of the volume of water with a second illumination

radiation at a second illumination wavelength which may be different from the first illumination wavelength. Accordingly, in some embodiments, the method further comprises illuminating the volume of water with a second illumination radiation comprising a second illumination wavelength. Yet alternatively, the  
5 detected radiation, based on which the process determines whether zooplankton is present within the volume of water, is detected without active illumination of the volume of water.

In some embodiments, detecting radiation received from the volume of water  
10 comprises recording blue autofluorescence from zooplankton within the volume of water, and determining whether zooplankton is present within the volume of water based on the recorded blue fluorescence. For example zooplankton may be determined to be present within the volume of water when the recorded blue fluorescence exceeds a threshold, e.g. a detection threshold or a predetermined  
15 threshold.

The blue autofluorescence may be recorded by detecting fluorescent light. Accordingly, the detected radiation may include fluorescent light, in particular in the blue range of the visible spectrum. The detected fluorescent light may be  
20 fluorescent light emitted from zooplankton in the volume of water responsive to being illuminated by the first illumination radiation and/or by the second illumination radiation.

Blue autofluorescence from zooplankton includes the innate fluorescence having  
25 wavelengths in the blue range of the visible spectrum. In some embodiments, the blue autofluorescence is measured in a wavelength range having end points within the range 400 - 650 nm, preferably within the range 450 - 600 nm, most preferably within the range 500 - 550 nm.

30 In some embodiments, identifying whether the zooplankton is a non-phytoplankton-eating zooplankton comprises identifying non-phytoplankton-eating zooplankton from the amount of autofluorescence from chlorophyll and from the recorded blue autofluorescence, such as from the amount of autofluorescence from chlorophyll relative to the recorded blue autofluorescence.

In some embodiments, identifying whether the zooplankton is a non-phytoplankton-eating zooplankton comprises:

- calculating a ratio of the measured amount of autofluorescence from chlorophyll from the zooplankton and a measured amount of the recorded blue autofluorescence from the zooplankton, and/or
  - determining a first proportionality parameter for the measured amount of autofluorescence from chlorophyll from the zooplankton and determining a second proportionality parameter for a measured amount of the blue autofluorescence from the zooplankton, and
- wherein identifying whether the zooplankton is a non-phytoplankton-eating zooplankton is further based on the calculated ratio and/or is further based on the determined proportionality parameters.

As mentioned above, in some embodiments, the method further comprises illuminating the volume of water with a second illumination radiation comprising a second illumination wavelength, in particular a second illumination wavelength different from the first illumination wavelength.

In particular, in some embodiments, detecting radiation received from the volume of water comprises recording an amount of reflected radiation from zooplankton within the volume of water, the reflected radiation comprising reflected radiation of the second illumination wavelength, and determining whether zooplankton is present within the volume of water is based on the recorded reflected radiation. In particular, the reflected radiation may be radiation reflected from zooplankton within the body of water responsive to being illuminated with the second illumination radiation.

In some embodiments, the first and second illumination radiation illuminate the volume of water at separate times, which may facilitate distinction between the reflected radiation and fluorescent light, even when the second illumination wavelength corresponds to, or overlaps with, the wavelength of the fluorescent light that is indicative of autofluorescence from chlorophyll. At separate times means that the first and second illumination radiation do not illuminate the water at the same time, at least not when the reflected radiation or the fluorescent light is detected. For example, the illumination may be alternated at a sufficiently high

rate so as to allow the detected radiation at the respective wavelength to be spatially and temporally correlated with each other. For example, a sufficiently high rate may depend on the typical speed of movement of the zooplankton to be detected, e.g. such that the zooplankton only moves sufficiently little between the illumination with the respective wavelengths so as to allow the received signal to be correlated with each other. It will be appreciated that similar considerations apply for the temporal resolution, e.g. the frame rate, of the detector.

In some embodiments, the second illumination wavelength has a value within the wavelength range 650 - 750 nm, preferably within the range 650 - 725 nm, most preferably within the range 650 - 700 nm. Accordingly, when the second illumination wavelength corresponds to the wavelength at which autofluorescence from chlorophyll is measured, detection of the reflected radiation and measurement of the autofluorescence from chlorophyll may be performed by a simplified detector unit. In particular, in this case the detector unit only needs to be capable of detection radiation within a single wavelength range.

In some embodiments, identifying whether the zooplankton is a non-phytoplankton-eating zooplankton comprises identifying non-phytoplankton-eating zooplankton from the amount of autofluorescence from chlorophyll and from the recorded reflected radiation, e.g. from the amount of autofluorescence from chlorophyll relative to the recorded reflected radiation. In some embodiments, identifying whether the zooplankton is a non-phytoplankton-eating zooplankton comprises calculating a ratio of the measured amount of autofluorescence from chlorophyll from the zooplankton and the recorded reflected radiation from the zooplankton.

In some embodiments, a suitable method may be employed to determine not only whether, but also where zooplankton are present within the volume of water, so that following this determination it is possible to measure an amount of autofluorescence from chlorophyll and to detect radiation (fluorescent or reflected) from individual zooplankton. For example, image processing of a captured image of the volume of water may be used to identify objects within the image. The image processing may even allow to recognize zooplankton within the captured image. The process may then determine whether the detected objects or

the detected zooplankton are non-phytoplankton-eating zooplankton from a measured amount of autofluorescence from chlorophyll emitted from the detected objects, e.g. alone or from a measured amount of autofluorescence from chlorophyll relative to a recorded amount of other fluorescent light and/or reflected light.

5

Generally, detecting individual zooplankton, i.e. individual organisms, and detecting autofluorescence from chlorophyll from those detected zooplankton provides an accurate determination as to whether or not the detected zooplankton is a phytoplankton-eating zooplankton. In particular this may be achieved by  
10 spatially and temporally correlating the measured amount of autofluorescence from chlorophyll with the detected radiation based on which the presence of zooplankton is determined. In particular, a spatially and time-resolved detection may be performed by one or more suitable image sensors, e.g. a 1D or 2D image sensors that allow time resolved measurements.

15

Alternatively, measuring an amount of autofluorescence from chlorophyll, and detecting radiation (fluorescent or reflected) from zooplankton within the volume of water may be done by measuring fluorescence and detecting radiation from the entire volume of water.

20

In any event, the method may comprise (subsequently or concurrently) repeating the illuminating, detecting, measuring and identifying acts in respect of different volumes of water so as to obtain improved statistics of the results.

25 In some embodiments, the method further comprises reporting to a system or to a user whether one or more non-phytoplankton-eating zooplankton has been identified within the volume of water and/or an amount of non-phytoplankton-eating zooplankton having been identified within the volume of water, e.g. during a measurement period and/or as a function of time. For example, reporting to a  
30 user may comprise displaying on a display an indication of whether non-phytoplankton-eating zooplankton has been identified and/or of an amount of identified non-phytoplankton-eating zooplankton. Alternatively or additionally other forms of output may be used, such as a print-out. Reporting to a system may comprise storing the measurement results on a portable storage device or  
35 communicating the measurement results via another suitable data communication

interface, e.g. to another computer program or functional unit and/or to an external data processing system.

In another aspect, an apparatus for detecting freely swimming non-  
5 phytoplankton-eating zooplankton is disclosed. The apparatus comprises:

- a light source unit adapted to emit a first illumination radiation comprising a first illumination wavelength, the first illumination radiation being capable of inducing autofluorescence of chlorophyll;
- one or more optical elements configured to illuminate a volume of  
10 water with the first illumination radiation;
- a detector unit arranged to detect radiation from the volume of water;
- a processing unit configured to determine whether zooplankton is present within the volume of water based on the detected radiation;

wherein the detector unit is further configured to measure an amount of  
15 autofluorescence from chlorophyll from a zooplankton present within the volume of water, and the processing unit is further configured to determine whether a zooplankton within the volume of water is a non-phytoplankton-eating zooplankton based on the measured amount of autofluorescence from chlorophyll.

20 In some embodiments, the light source unit comprises a laser. Alternatively or additionally, other light sources may be comprised within the light source unit, e.g. one or more light-emitting diodes.

In some embodiments, the first illumination wavelength has a value within the  
25 range 365 nm - 740 nm, preferably within the range 365 nm - 650 nm.

When the first illumination wavelength has a value within the preferred range of 365 nm - 650 nm then the autofluorescence from chlorophyll may be measured while the volume of water is being illuminated by the first illumination radiation by  
30 using a suitable bandpass filter in the detector unit so as to filter out the first illumination radiation. Alternatively, if the first illumination wavelength has a value above a certain value such as e.g. 650 nm then other components and/or method steps may be needed to measure the autofluorescence from chlorophyll such as e.g. one or more advanced filters and/or switching the light source on and off.

In some embodiments, the detector unit is configured to record blue autofluorescence from zooplankton within the volume of water.

In particular, in some embodiments, the detector unit is configured to record blue autofluorescence in a wavelength range having end points within the range 400 - 5 650 nm, preferably within the range 450 - 600 nm, most preferably within the range 500 - 550 nm.

In some embodiments, the processing unit is further configured to:

- 10 - calculate a ratio of the measured amount of autofluorescence from chlorophyll from the zooplankton and a measured amount of the recorded blue autofluorescence from the zooplankton, and/or
- determine a first proportionality parameter for the measured amount of autofluorescence from chlorophyll from the zooplankton and
- 15 determine a second proportionality parameter for the blue autofluorescence from the zooplankton, and

wherein the processing unit is further configured to determine whether the zooplankton is a non-phytoplankton-eating zooplankton based on the calculated ratio and/or based on the determined proportionality parameters.

20

In some embodiments, the light source unit is further configured to emit a second illumination radiation comprising a second illumination wavelength, the detector unit is further configured to record an amount of reflected radiation from zooplankton within the volume of water, the reflected radiation comprises 25 reflected radiation of the second illumination wavelength, and the processing unit is further configured to determine whether zooplankton is present within the volume of water based on the recorded reflected radiation. It will be appreciated that the light source unit may include a first light source adapted to emit the first illumination radiation and a second light source adapted to emit the second 30 illumination radiation. Alternatively, the light source unit may include a single light source adapted to emit the first illumination radiation and the second illumination radiation.

In some embodiments, the light source unit is further configured to illuminate the 35 volume of water with the first and second illumination radiation at separate times.

In some embodiments, the second illumination wavelength has a value within the wavelength range 650 - 750 nm, preferably within the range 650 - 725 nm, most preferably within the range 650 - 700 nm.

5

In some embodiments, the detector unit comprises a detector and collection optics.

In some embodiments, the detector is placed in Scheimpflug angle with respect to  
10 the collection optics, the first and second illumination radiation are emitted by one or more light sources, the one or more light sources are co-aligned and the one or more light sources are arranged to lie in the Scheimpflug focal plane.

Generally, alternatively or additionally, the detector unit may include other types  
15 of light detectors, e.g. one or more photodiodes, and array of light sensitive elements, a quadrant detector, or the like. In some embodiments, the detector unit comprises more than one detector adapted to detect the autofluorescence light and the detected radiation, respectively. In other embodiments, the detector unit comprises only a single detector, e.g. a detector sensitive at different wavelength  
20 ranges or at a common wavelength range of reflected radiation and autofluorescent light from chlorophyll.

In some embodiments, the processing unit is further configured to:

- calculate a ratio of the measured amount of autofluorescence from  
25 chlorophyll from the zooplankton and the recorded reflected radiation from the zooplankton, and
- determine whether the zooplankton is a non-phytoplankton-eating zooplankton based on the calculated ratio.

30 Here and in the following, the term processing unit is intended to comprise any circuit and/or device suitably adapted to perform the functions described herein. In particular, the term processing unit comprises a general- or special-purpose programmable microprocessor, such as a central processing unit (CPU) of a computer or of another data processing system, a digital signal processor (DSP),  
35 an application specific integrated circuits (ASIC), a programmable logic arrays

(PLA), a field programmable gate array (FPGA), a special purpose electronic circuit, etc., or a combination thereof. A processing unit may be embodied as a single processor or as a distributed system including multiple processors, e.g. a client-server system, a cloud based system, etc.

5

In some embodiments, the detector unit and/ or the light source unit may be a submersible unit configured to be submerged into the body of water and to operate while being submerged in the body of water. The detector unit and the light source unit may be combined into a single housing, in particular a single  
10 submersible housing. Alternatively, the detector unit and the light source unit may be accommodated in separate respective housings, in particular submersible housings. For example, the detector unit and/ or the light source unit may, during operation be submerged into an industrial salmon farm, e.g. mounted to a sea cage for accommodating salmon. In some embodiments, the detector unit and/or  
15 the light source unit is configured to be mounted above the body of water, e.g. floating on the surface of the body of water or mounted elevated above the surface of the body of water.

Accordingly, the present disclosure also relates to a sea cage for accommodating  
20 salmon, the sea cage comprising an apparatus disclosed herein. The apparatus, in particular the detector unit and/or the light source unit, may be attached to the cage and/or otherwise mounted relative to the cage. The apparatus, in particular the detector unit and/or the light source unit, may be configured to detect non-phytoplankton-eating zooplankton, in particular freely swimming salmon lice,  
25 inside the cage and/or in a proximity of the cage.

In some embodiments, the apparatus comprises a container defining a sample volume. The container may comprise a sample inlet and a sample outlet. The outlet may be separate from the inlet or the inlet and outlet may be provided as a  
30 combined inlet and outlet port. The apparatus may further comprise a pump for creating a flow of water through the container or a drive mechanism for moving the container through a body of water so as to exchange the contents of the container. The apparatus may comprise a light source unit and/or a detector unit such that the light source unit illuminates the interior of the container and/or such  
35 that the detector unit receives light from organisms inside the container. The

apparatus may further comprise a liquid sample filter, e.g. an oscillating mesh filter, for filtering out particles smaller than the zooplankton to be detected. For example, an oscillating mesh filter may be configured to block objects of the size of the zooplankton to be detected and to allow smaller particles to pass. The  
5 blocked objects may be led to a measurement chamber, e.g. by a suitable pump mechanism. Accordingly, background fluorescence, respectively for the cyan band from dissolved organic matter and the red band due to chlorophyll from algae in the water may be reduced.

10 According to another aspect, a computer program comprises program code adapted to cause, when executed by a data processing system, the data processing system to perform at least the determining and identifying acts of one or more of the methods described herein. The computer program may be embodied as a computer-readable medium, such as a CD-ROM, DVD, optical disc,  
15 memory card, flash memory, magnetic storage device, floppy disk, hard disk, etc. having stored thereon the computer program. According to one aspect, a computer-readable medium has stored thereon instructions which, when executed by one or more processing units, cause the processing unit to perform one or more steps of an embodiment of the process described herein.

20

Additional features and advantages will be made apparent from the following detailed description of embodiments that proceeds with reference to the accompanying drawings.

## 25 BRIEF DESCRIPTION OF THE FIGURES

FIG. 1A shows an example of an apparatus for detecting freely swimming non-phytoplankton-eating zooplankton.

FIG. 1B shows an example of an apparatus for detecting freely swimming non-  
30 phytoplankton-eating zooplankton using a Scheimpflug lidar setup.

FIG. 2 shows fluorescence images of five zooplankton species.

FIG. 3 shows an average fluorescence spectrum of larval stage salmon lice (*35 salmonis*), which is a non-phytoplankton-eating zooplankton.

FIG. 4 shows average fluorescence spectra of five zooplankton species, four of which are phytoplankton-eating and one of which is non-phytoplankton-eating.

5 FIG. 5 shows graphs of measured fluorescence spectra (full lines) of three species of zooplankton. The measured spectra are fitted by two reference spectra (dashed lines) to determine the proportionality constants  $a_{lice}$  and  $a_{chl}$ .

FIG. 6 shows a scatter plot of the ratio of the proportionality constants  $a_{chl}$  and  
10  $a_{lice}$  versus  $a_{cm}$ .

FIG. 7 shows a fluorescence spectrum of a phytoplankton-eating zooplankton with indication of 50 nm wide wavelength bands used for dual fluorescence band analysis.

15

FIG. 8 shows a scatter plot of the ratio of  $\sum I^{*red}$ , the summed red fluorescence band intensity, and  $\sum I^{*blue}$ , the summed blue fluorescence band intensity, versus  $\sum I^{*red}$ .

20 FIG. 9 schematically illustrates an example of an apparatus for detecting non-phytoplankton-eating zooplankton, in particular salmon lice.

FIG. 10 illustrates an example of a process for detecting freely swimming non-phytoplankton-eating zooplankton.

25

FIG. 11 illustrates typical spectra of *L. salmonis* and *A. tonsa*.

FIG. 12 shows a cyan-versus-red-signal scatter plot for a single individual of each species from two separate measurements.

30

FIG. 13 illustrates measurements performed for 6 hours on 50 individuals of each species in two separate single-species cultures.

FIG. 14 illustrates measurements on single-species and mixed-species cultures of  
35 two species in different ratios.

## DETAILED DESCRIPTION

FIG. 1A shows an example of an apparatus for detecting freely swimming non-phytoplankton-eating zooplankton. A detector unit 10, which may comprise one or more detectors, receives radiation from a volume of water 14, which is or has been illuminated by illumination radiation from a light source unit 16. The illumination radiation comprises an illumination wavelength capable of inducing autofluorescence of chlorophyll, i.e. having a value within the range 365 nm - 740 nm, but preferably within the range 365 - 650 nm. The light source unit may comprise e.g. a 405 nm or 445 nm high power (1 - 3W) laser and may be operated in an on/off modulation, which allows for elimination of background illumination. Alternatively, or additionally, the apparatus may comprise other types of light sources, including light sources operating at other wavelengths suitable for inducing autofluorescence from chlorophyll and/or blue autofluorescence from zooplankton as described below. The detector unit 10 may be positioned 0.5 - 1 meter from the volume of water 14.

Each detector of the detector unit 10 may have a band pass filter 18 in front of it so as to only measure light in a relevant part of the detectable spectrum. Zooplankton is autofluorescent and will emit light having wavelengths in the blue/cyan part of the visible spectrum when exposed to illumination radiation of an appropriate wavelength. The blue autofluorescence from zooplankton could be measured in a wavelength range having end points e.g. within the range 400 - 650 nm, or 450 - 600 nm, or, most preferably, 500 - 550 nm.

A measurement of this blue autofluorescence from zooplankton, see FIG. 2, induced by illumination radiation from the light source unit 16 may be used to determine whether zooplankton is present within the volume of water 14. This determination may be done by a processing unit 19 using e.g. a particle finding algorithm, such as e.g. a particle detection algorithm, an image segmentation algorithm or an event extraction algorithm. For example, to this end, the processing unit may process a captured image and/or other measurement results so as to detect one or more features and/or other indicators (e.g. clusters of blue/cyan pixels) indicative of the presence of one or more zooplankton in the volume of water, e.g. within a predetermined time period, a sliding window or the

like. The processing unit may identify individual organisms and/or determine an estimate of the population of organisms in the volume of water. As an alternative, other types of detection methods may be used to detect and determine the presence of zooplankton within the volume of water 14. The detection method 5 may be expanded by combining with alternative or additional image recognition processes to increase specificity.

Chlorophyll is likewise autofluorescent and will emit visible light having wavelengths in the red part of the visible spectrum. A measurement of this red 10 autofluorescence may be used to identify whether a zooplankton within the volume of water is a non-phytoplankton-eating zooplankton. The autofluorescence from chlorophyll could be measured in a wavelength range having end points e.g. within the range 650 - 750 nm, or 650 - 725 nm, or, most preferably, 650 - 700 nm.

15

In an apparatus which measures both blue and red autofluorescence, the detector unit 10 may comprise two detectors, which may share collection optics. In the case of shared collection optics, the incoming light may be split between the detectors using a shortpass dichroic mirror having a cut-off wavelength of e.g. 20 600 nm. Alternatively, a single detector may be used to measure both blue autofluorescence from zooplankton and red autofluorescence from chlorophyll using a suitable setup, e.g. suitable optical filters that can selectively be applied.

FIG. 1 B shows an example of an apparatus for detecting freely swimming non- 25 phytoplankton-eating zooplankton using a Scheimpflug lidar setup. A detector unit 10 comprises a detector, such as a single line array detector, which receives radiation from a volume of water 14 that is or has been illuminated by an illumination radiation from a light source unit 16. The imaging plane of the detector within the detector unit 10 is positioned in Scheimpflug angle,  $\Theta$ , with 30 respect to the lens plane of the collection optics 12. A light source within the light source unit 16 emits a first illumination radiation, which comprises a first illumination wavelength and a second illumination radiation, which comprises a second illumination wavelength. The light source unit 16 is arranged to lie in the Scheimpflug focal plane 15 and the object plane of the detector 10 is spatially 35 overlapped with the light source, i.e. the light source unit 16 and the object plane

of the detector 10 are both along the Scheimpflug focal plane 15. The light source unit 16 may comprise two lasers, which may be co-aligned. In this example the relevant probe volume, i.e. the volume of water 14 to be illuminated, is 0.5 - 1.5 meter in range from the detector unit.

5

The first illumination radiation is capable of inducing autofluorescence from chlorophyll, as described above for FIG. 1A, and may e.g. be a 405 nm or 445 nm high power (1 - 3W) laser. The second illumination radiation is capable of reflecting off of zooplankton and the second illumination wavelength may  
10 advantageously have a value within the range 650 - 700 nm, such as e.g. 660 nm. If using two light sources, comprised within the light source unit, they may be modulated so as to illuminate the volume of water 14 at separate times, e.g. one light source is on and another off at a given time. Alternatively, a sequence may be used where the first light source is on (and second light source off), then  
15 second light source on (and first light source off) and finally both light sources off. In the case of a single light source being used to generate both the first and the second illumination radiation, the sequence may be one, wherein the first illumination radiation is emitted, then the second illumination radiation is emitted and finally no radiation is emitted.

20

A bandpass filter 18 may be inserted in front of a detector within the detector unit 10. The bandpass filter 18 should allow both fluorescent light from chlorophyll and the light reflected off of zooplankton to pass. For example, if the second illumination wavelength has a value of 660 nm, the bandpass filter 18 may have  
25 cut-off wavelengths of 650 nm and 700 nm. In this way, a single detector within the detector unit 10 can be used to measure both the fluorescent light emitted by chlorophyll and the light reflected off of zooplankton within the volume of water 14.

30 If a laser is used to emit the first illumination radiation and this laser is a high power (1 - 3W) laser, a laser used to emit the second illumination radiation may advantageously be less powerful, such as a few hundred mW, so that the fluorescent and reflected light may be measured using the same exposure time.

The second illumination radiation reflected off of zooplankton may be used to determine whether zooplankton is present within the volume of water 14. This determination may be done by a processor 19 using e.g. a particle finding algorithm, such as e.g. a particle detection algorithm, an image segmentation algorithm or an event extraction algorithm, with examples being given above. Again, as an alternative, other types of detection methods may be used to detect and determine the presence of zooplankton within the volume of water 14, and the method may be expanded by combining the above with image recognition to increase specificity.

10

Alternatively, the method described above, wherein reflection of the second illumination radiation off of zooplankton is used to determine whether zooplankton is present within the volume of water, may be realised using an apparatus as shown in FIG. 1A, i.e. an apparatus not using a Scheimpflug lidar setup. A single detector within the detector unit 10 may advantageously be used to measure both the reflected light and the autofluorescence from chlorophyll, i.e. the single detector measures in a red wavelength band (e.g. using appropriate filters). In such an embodiment, the apparatus of FIG. 1A may e.g. be adapted by configuring the light source unit to emit both a first illumination radiation and a second illumination radiation, as described above. Any adaptations needed will be clear to any skilled person.

FIG. 2 shows fluorescence images of five different zooplankton species: a) *Temora longicornis*, b) *Oithona davisae*, c) *Calanus finmarchicus*, d) *Acartia tonsa*, and e) copepodid *Lepeophtheirus salmonis* (Salmon louse). The salmon louse is in its larval state in which it drifts in the water until it finds a host, preferentially salmon, on which to attach itself. Salmon lice are ectoparasites, i.e. parasites that live on the outside of its host, and they do not feed until attached to a host. Therefore, the fluorescence image of the salmon lice shows no fluorescence from chlorophyll.

In FIG. 2a) a dashed circle 20 shows the field-of-view of the detector, in this example a spectrometer, used for fluorescence measurements. In the colour version of the five images the zooplankton fluoresce in blue 24, while undigested chlorophyll within the zooplankton fluoresces in red 22. Salmon lice are non-

phytoplankton-eating and therefore no autofluorescence from chlorophyll is visible in the fluorescence image of a salmon louse in FIG. 2e). In FIG. 3 is shown an average fluorescence spectrum of four samples of salmon lice (*/. salmonis*) and it is apparent from this spectrum that primarily blue/cyan fluorescent light in a wavelength range around a peak centered at ~510 nm is emitted from the non-phytoplankton-eating zooplankton.

FIG. 4 shows average fluorescence spectra of the five zooplankton species shown in FIG. 2, where the averaging is done based on fluorescence spectra recorded on two to four individuals of each species. The salmon lice spectrum shows the single peak of blue fluorescence as seen in FIG. 3, whereas the spectra of the remaining four species, which are phytoplankton-eating, show peaks of red fluorescence from chlorophyll in addition to the single blue fluorescence peak. Thus, a measurement of fluorescent light from a zooplankton can be used to distinguish phytoplankton-eating zooplankton from non-phytoplankton-eating zooplankton such as e.g. salmon lice.

Different methods may be used to analyse the measured fluorescent light. FIG. 5 shows examples of two reference spectra (dashed lines) having been fitted to measured fluorescence spectra of a) salmon lice (*/. salmonis*), b) *O. davisae* and c) *C. finmarchicus* to determine two proportionality constants  $\alpha_{\text{lice}}$  and  $\alpha_{\text{chl}}$ . The proportionality constant  $\alpha_{\text{lice}}$  is used to scale a reference spectrum made from fluorescence spectra of salmon lice as representative of the blue autofluorescence from zooplankton, while the proportionality constant  $\alpha_{\text{chl}}$  is used to scale a chlorophyll reference spectrum. The fitting is based on linear spectral mixture analysis and the proportionality constants provide estimates of the abundance of each reference spectrum.

In the fits shown in FIG. 5 two reference spectra were created from averaged salmon lice fluorescence spectra and averaged chlorophyll fluorescence spectra, i.e. the latter being recorded without zooplankton present. The reference spectra were given proportionality constants  $\alpha_{\text{lice}}$  and  $\alpha_{\text{chl}}$ , respectively, and had a restraint of non-negativity, i.e.  $\alpha_{\text{lice}}$  and  $\alpha_{\text{chl}}$  were not allowed to be negative. In the case of the salmon louse fluorescence spectrum in FIG. 5a), the fit results in a value of  $\alpha_{\text{chl}}$  that is close to zero, whereas the fits of fluorescence spectra from

phytoplankton-eating zooplankton in FIG. 5b) and c) result in a comparatively large value of  $\alpha_{chl}$ . The dashed lines are the resulting reference chlorophyll and salmon lice spectra scaled with the proportionality constants obtained.

- 5 In a proof-of-concept visualization, the obtained proportionality constants may be plotted in a scatter plot of the ratio of the proportionality constants  $\alpha_{chl}$  and  $\alpha_{once}$ , i.e.  $\alpha_{chl}$  divided by  $\alpha_{once}$ , versus  $\alpha_{chl}$  on a double logarithmic scale as shown in FIG. 6. Each circled set of points represent data obtained from a species of zooplankton and it is apparent that the non-phytoplankton-eating zooplankton 60 is
- 10 distinguishable within the plot from the phytoplankton-eating zooplankton 62. Accordingly, the proportionality constants may be used to determine whether one or more zooplankton is a non-phytoplankton-eating zooplankton, for example by the processing unit having one or more algorithms thereon configured to use the proportionality constants and/or one or more values calculated from the
- 15 proportionality constants to ascertain whether a zooplankton belongs in a defined set of points or not, e.g. based on a comparison with predetermined upper and/or lower thresholds.

Another method that may be employed to identify non-phytoplankton-eating

20 zooplankton is dual fluorescence band analysis, wherein the blue autofluorescence from zooplankton and the red autofluorescence from chlorophyll are recorded in separate wavelength bands, i.e. separate wavelength ranges. To measure an amount of fluorescence within the wavelength bands, a possible apparatus is one in which the detector unit 10 measures both blue autofluorescence from

25 zooplankton and red autofluorescence from chlorophyll within a wavelength band of e.g. 50 nm. The wavelength bands may be of different sizes, but as an example the blue fluorescence may be measured within a wavelength band of 500 - 550 nm, while the red autofluorescence may be measured within a wavelength band of 650 - 700 nm.

30

FIG. 7 shows, as an example, a fluorescence spectrum with two 50 nm-wide wavelength bands indicated: a blue wavelength band having end points of 490 nm and 540 nm and a red wavelength band having end points of 661 nm and 711 nm. The wavelength bands may, of course, have a width different from 50 nm and

35 be centered at wavelengths other than those given in the example. The 50 nm

bandwidth was chosen for the analysis as this width covers most of the peak in intensity from chlorophyll fluorescence around 670 nm. For dual fluorescence band analysis the intensity need not be recorded as a function of wavelength, but may merely be recorded as an accumulated intensity or counts within each of the 5 wavelength bands. In FIG. 7 the wavelength band are shown as extracted from a wide fluorescence spectrum, but for the dual fluorescence band analysis only the intensities within the spectral bands are required and the apparatus used to measure the fluorescence may have components such as e.g. bandpass filters, so as to only measure the intensities within the spectral bands. Accordingly, a 10 simplified detection unit may be used and no spectrometer is required.

In FIG. 8 is shown a double logarithmic scatter plot of the ratio of  $\sum I^*_{red}$ , the summed red fluorescence band intensity, and  $\sum I^*_{blue}$ , the summed blue fluorescence band intensity, versus  $\sum I^*_{red}$ . Again, the distribution of scatter points 15 based on salmon lice measurements 80 are distinguishable from those of the phytoplankton-eating species 82. This demonstrates that dual fluorescent band analysis, wherein sums of the intensity within two wavelength bands are used, holds sufficient information to distinguish a non-phytoplankton-eating zooplankton, here salmon lice, from phytoplankton-eating zooplankton.

20

Similar to the scatter plots shown in FIGs. 6 and 8, an analysis of the recorded reflected light and fluorescent light using an apparatus with a Scheimpflug lidar setup, as shown in FIG. 1B, may be made. In this case, a scatter plot of the ratio of the fluorescent light and reflected light versus the fluorescent light may be used 25 to distinguish non-phytoplankton-eating zooplankton from phytoplankton-eating zooplankton.

FIG. 9 schematically illustrates an example of an apparatus for detecting non-phytoplankton-eating zooplankton, in particular salmon lice. In this example, the 30 apparatus is a dual-band fluorescence detection apparatus. The apparatus is configured to detect non-phytoplankton-eating zooplankton in a body of water 100, such as in a container at least partially filled with water.

The apparatus comprises a light source for illuminating at least a portion 400 of 35 the body of water 100. In this example, the light source includes a diode laser

source LD emitting a collimated laser beam into the volume of water. In this example, the diode laser source includes a violet 410 nm, 500 mW laser diode with beam size 3 mm x 5 mm. Accordingly, in this example, the illuminated portion 400 of the body of water 100 is defined by the beam path and cross section of the collimated laser beam. In other examples, where other illumination systems are used, the illuminated portion may have a different shape and size.

The apparatus further comprises a detector 200. In the example of FIG. 9, the detector and the laser LD are positioned outside the container. To this end, the container has at least partially transparent walls. It will be appreciated, however, that, in other embodiments, the detector and/or the light source may be submerged into the body of water, floating at the surface of the body of water and/or be mounted above the body of water. For example, the light source may be submerged into the body of water and the detector unit may be floating at the surface of the body of water. To this end the light source and/or the detector may be accommodated in suitable housings.

The detector includes one or more optical elements, e.g. one or more lenses L and one or more mirrors DM, to image the volume illuminated by the light source onto respective sensor arrays LA1 and LA2. The detector includes two linear image sensor arrays and the optical elements are arranged to image the fluorescent light from animals in the illuminated volume onto each of the image sensor arrays. In the example of FIG. 9, the optical elements include an achromatic doublet lens (Thorlabs, AC508-100-A-ML), L, with a focal length of 100 mm configured to collect induced fluorescence from animals 300 in the illuminated volume at an angle of 90° relative to the direction of incidence of the laser beam. The image sensors are two identical 16-bit linear array detectors (Synertronic Designs, LineScan-I-Gen2), LA1 and LA2, using a longpass dichroic mirror (Thorlabs, DMLP550L), DM, with a cutoff wavelength of 550 nm. For each detector, a filter set, FS1 and FS2, respectively, is arranged to select the relevant fluorescence band and to eliminate elastic light from the laser. Filter FS1 contains an OD5 longpass filter with a cutoff wavelength of 500 nm (Thorlabs, FELH0500) and an OD4 bandpass filter of 50 nm bandwidth and center wavelength at 525 nm (Edmund Optics #86-951). Filter FS2 contains an OD6 longpass filter with a cutoff

wavelength of 550 nm (Thorlabs, FEL0550) and an OD4 bandpass filter of 50 nm bandwidth and center wavelength at 700 nm (Edmund Optics #84-787).

The magnification of 1/10 is chosen so the image of a salmon louse covers a minimum of 5 pixels, and most of the laser beam is imaged within the 200 pm height of the detector pixel (pixel size is 14 pm x 200 pm). Spatially overlapping the field-of-view of the two detectors was done by imaging a fluorescent bead that contained fluorescence in both bands. The wavelength bands are chosen based on the fluorescence spectra of the zooplankton to be detected.

10 It will be appreciated that other embodiments of an apparatus may include alternative or additional types of optical elements, detectors, light sources and/or filters. It will further be appreciated that the apparatus may comprise or be operationally coupled to a signal processing unit and/or a data processing unit for processing the sensor signals and/or for analysing the collected data to detect  
15 freely swimming non-phytoplankton-eating zooplankton, e.g. by performing the process of FIG. 10.

FIG. 10 illustrates an example of a process for detecting freely swimming non-phytoplankton-eating zooplankton.

20

In an initial step S0, detector data is acquired e.g. using the apparatus of FIG. 9. The detector data may include spatially and temporally resolved light intensities at least in respective wavelength bands in which autofluorescence of chlorophyll and fluorescence of the zooplankton, respectively, occur. For example, when using the  
25 apparatus of FIG. 9, the sensor data from each of the 1D sensor arrays may be represented at a sequence of one-dimensional images. The sequence of one-dimensional images may e.g. be represented as 2D arrays where each row of the array represents one of the sequence of 1D images. The apparatus of FIG. 9 may thus output a series of files, each file representing a 2D array representing a  
30 sequence of 1D images. The first series of files corresponds to the data from a first one of the two 1D sensor arrays of FIG. 9 (sensor LA1) - in the following referred to as the cyan channel. The second series of files corresponds to the data from the other one of the two 1D sensor arrays of FIG. 9 (sensor LA2) - in the following referred to as the red channel. Other embodiments may use one or more

2D image sensors, and the the sensor data may be represented as one or more sequences of 2D images, e.g. as a sequence of 2D image files or as a 3D array.

In subsequent step 1 the acquired sensor data is pre-processed, e.g. so as to  
5 remove the background fluorescence of the water. This may be done by taking the median along the time axis (for every pixel position of each 2D array) and by subtracting the median from the raw signal. Using the median rather than the time average was found to provide a higher robustness to high-intensity events. This background subtraction assumes that the desired events are few and short  
10 enough that each pixel is dominated by the background fluorescence of filtered seawater. In other situations other background suppression methods may be used.

In subsequent step 2, the process performs an event detection on the  
15 preprocessed 2D arrays. For example, events may be determined by a method similar to the one described in E. Malmqvist, S. Jansson, S. Torok, and M. Brydegaard, "Effective parameterization of laser radar observations of atmospheric fauna," IEEE J. Sel. Top. Quantum Electron., 22, 327-334 (2006). In particular, a binary mask may be created based on the cyan channel to pick out  
20 each event. To create this mask, a threshold may be applied to the 2D arrays of the cyan channel. The threshold used may e.g. be  $18 \cdot (2 \cdot \text{median} - \text{minimum})$ , which has been found to be high enough to remove the background and particles that are too small to be animals. However, other embodiments may use other thresholds. The resulting signal may then be eroded so as to remove noise peaks.  
25 Subsequently, the 2D arrays may be dilated so as to contain all the pixels within the event. Finally, the mask may be multiplied with the original signal in both channels. It will be appreciated that other embodiments may utilise other event detection algorithms known as such in the art.

30 In subsequent step 3, the process determines the signal strength of each detected event, for each channel. The signal strength of the event may be found as the sum along the pixel axis averaged over time.

In subsequent step 4, the detected events are processed so as to discard events that are saturated, are too narrow (having a mask having a width smaller than a  
35 minimum threshold, e.g. smaller than 6 pixels) or have a mean pixel signal

strength below a minimum threshold, e.g. below 1.5 times the standard deviation of the background.

In subsequent step S5, the process determines, for each of the remaining events, 5 whether the event relates to a non-phytoplakton-eating zooplankton or not. In particular, this determination may be based on the relative signal strengths in the two channels associated with said event, e.g. as described below. In particular, the determination may be based on a classification of the detected events based on the signal strengths in the respective wavelength bands. The classification may 10 e.g. be performed by defining a suitable classifier, e.g. a boundary (e.g. a linear or non-linear boundary) in a two-dimensional space spanned by the respected signal strengths. The classifier may be determined based on a set of calibration/training data, in particular by a set of detected events associated with known organisms. Other examples of classifiers may include a machine-learning model trained on a 15 set of training data.

#### Examples:

The apparatus of FIG. 9 was used to detect salmon lice and distinguish salmon 20 lice from algae-eating zooplankton.

FIG. 11 illustrates typical spectra of *L. salmonis* and *A. tonsa*. In FIG. 11, the wavelength bands defined by the filters FS1 and FS2 of the apparatus of FIG. 9 are illustrated as shaded bands labelled FS1 and FS2, respectively. The larvae of 25 salmon lice do not eat phytoplankton while *A. tonsa* are algae-eating.

*L. salmonis* in the parasitic copepodite life stage were supplied by ILab and were kept at 10°C. The *A. tonsa* were supplied by DTU Aqua in their adult life stage and were kept at 18°C. A species from the *Acartia* genus was chosen to show the 30 identification capabilities of the system since this genus is abundant in the North Sea and has approximately the same size as the salmon lice copepodites. Adult *A. tonsa* are 1.2 mm long while copepodite *L. salmonis* are 0.8 mm long on average. The animals used in this example were are alive and swam freely in the water during measurements.

The experiments were performed using a framerate of 200 Hz, and an exposure time of 4.98 ms, which was found to best utilize the dynamic range (16-bit) of the detectors. The linear array detectors were set to output a file every 30 seconds, resulting in data files of dimensions  $M \times N$ , where  $M = 6000$  is the number of  
5 acquisitions in time and  $N = 1000$  is the number of pixels. The data files were processed on a computer to find events. Each event is defined by the time and pixel positions associated with an organism detected in the illuminated portion of the body of water defined by the laser beam. The event detection was performed as described in connection with FIG. 10.

10

To eliminate the effect of potential variation in fluorescence between individuals of the same species, measurements were initially carried out on a single individual from each species in filtered seawater. Each measurement was conducted over 3-4 hours and the results show that the two species have significantly different  
15 distributions in a scatter plot where each detected event is plotted in a two-dimensional space spanned by the signal strengths in the red and cyan channels, as illustrated in FIG. 12.

FIG. 12 shows a cyan-versus-red-signal scatter plot for a single individual of each  
20 species from two separate measurements. Each point corresponds to an event when that individual passes through the 410 nm excitation beam.

*A. tonsa* shows a stronger red fluorescence as expected from chlorophyll. However, its gut is empty for the majority of the measurements, since *A. tonsa*  
25 clears its gut within 30-60 minutes of being in filtered seawater. This suggests that the red fluorescence does not stem exclusively from the undigested chlorophyll in the gut, as there is no significant variation in the cyan over red fluorescence ratio over time. Thus, at least part of the red fluorescence signal must stem from tissue signal. The distribution from each individual shows a linear  
30 relation between the signal strengths of the two channels. It is also expected in *A. tonsa* since it was starved for most of this experiment, so there would be no significant variation due to gut content. The results in FIG. 12 show that there is a significant variation of signal strength within each species. This variation stems from the different positions of the zooplankton within the laser beam (i.e. different  
35 excitation intensities) and how well in focus the animal is. It was observed that

the animals from both species tend to react to the laser beam by jumping when they reached the beam center. Thus, the measurements appear alter the animals' natural behavior, which is not considered an issue for the proposed application but is worth mentioning. The absence of significant variation in the cyan-to-red signal ratio of the same individual over time suggests the applicability of the method, which does not seem to affect the health of the animals. Unlike in the present example where the animals swim across the laser beam several times, excessive exposure of any individual zooplankton is less likely in a field measurement or in a submersible fluorosensor with a flow system.

10

Next, measurements were carried out on monoculture samples of each species, with each sample containing approximately 50 individuals. The system was kept running for about 6 hours to collect a big dataset for generating a classifier. The salmon lice were measured in filtered seawater, while *A. tonsa* were measured in water that contained algae (*Rhodomonas salina*). Over the measurement period, the algae gradually settled. This means there were a combination of fed and starved individuals over the measurement period, which is desirable for training a classifier, since both starved and fed animals will be expected in the field. Animals were considered fed during the first 3 hours and starved during the last 3 hours. More than 1000 events were gathered from each species. The results are presented in FIG. 13.

FIG. 13 illustrates measurements performed for 6 hours on 50 individuals of each species in two separate single-species cultures. The *A. tonsa* measurements are further divided into fed animals (algae present in the water) and starved animals (low algae concentration for >30 minutes). FIG. 13 (a) shows a histogram of the training set consisting of 90% of the events. The dashed black line indicates a dividing line between the two groups,  $y_S = 15$ , i.e. the dashed line represents a linear classifier determined from the training set. FIG. 13 (b) shows a scatter plot of the remaining test set consisting of 10% of the events. The dashed black line represents a linear classifier determined from the training set. FIG. 13(c) shows the resulting confusion matrix of the test set when using a linear classifier determined from the training set.

A test set was generated by selecting every 10<sup>th</sup> event, with the remaining 90% of the total events then used as a training set. The training set is shown in a histogram of the ratio of cyan to red fluorescence signal strength in FIG. 13(a). It is evident from the figure that the two species have distinct distributions, and that the cyan/red ratio is higher for starved *A. tonsa* than for fed ones. This indicates that the chlorophyll from undigested chlorophyll in the gut further increases the red signal from the tissue and leads to a larger variation in cyan-to-red ratio,  $\gamma$ , for *A. tonsa*, than in the single individual experiment. Based on the distributions of the training set, a simple classification algorithm was created. The classifier is based on the cyan-to-red ratio. The slope of the dividing line between the two groups is the ratio,  $\gamma_s$ , that is equidistant from the mean cyan-to-red ratio of the two species normalized to their standard deviation. This is given by the equation:

$$\gamma_s = \frac{\mu_{AT}\sigma_{LS} + \mu_{LS}\sigma_{AT}}{\sigma_{AT} + \sigma_{LS}} \quad (1)$$

where  $\mu$  and  $\sigma$  are the mean and the standard deviation, respectively, of the  $\gamma$  of each species indicated by subscripts, AT (for *A. tonsa*) and LS (for *L. salmonis*).  $\gamma_s$  is found to be 15 and is indicated by the black dashed line in FIG. 13(a). This classifier was then used to predict the groups of the events in the test set, which is shown in the scatter plot in FIG. 13(b). The number of predicted individuals belonging to a species as compared to the actual number is shown in FIG. 13(c), and these are used to calculate the performance of the system in distinguishing *L. salmonis* from *A. tonsa*. The sensitivity is calculated as the ratio of the number of salmon lice correctly identified to the actual number of salmon lice. Similarly, specificity is calculated as the ratio of the number of *A. tonsa* correctly identified and the actual number of *A. tonsa*. It is found that the system has a sensitivity of 98.9% and a specificity of 94.1%. This shows that the system can distinguish these two species with high accuracy. The accuracy can be increased by using harsher filtering on the SNR, with the downside of reducing the number of events. Finally, a measurement was conducted with mixed-culture samples of the two species in different ratios. In this case, the actual species of each event is not known. The results are shown in FIG. 14.

FIG. 14 illustrates measurements on single-species and mixed-species cultures of the two species in different ratios as indicated on the respective histograms. LS is

the number of *L. salmonis* and AT is the number of *A. tonsa* in each measurement. The colors indicate which species the classifier has predicted for the events (blue = LS and red = AT).

5 Each histogram represents a different mix ratio. The predicted species, with the dashed line indicating the dividing line  $\gamma^s$ , are shown: Events to the left of the dashed line are predicted as *A. tonsa* and events on the right of the dashed line are predicted as *L. salmonis*. From the two single-species measurements (top and bottom histograms), we find that the sensitivity is 93.9% while the specificity is  
10 94.7%. That is, the sensitivity is slightly lower than the previous measurement but still high. The predicted distributions of species in the mixed-culture measurements are calculated to see if it follows the known distributions. It is found that for actual concentrations of 83%, 50%, and 17% salmon lice, the predicted percentage of salmon lice are 91%, 34%, and 19%, respectively. The  
15 predicted distributions follow the trend that a decreasing percentage of salmon lice shows as a decreasing percentage of predicted salmon lice. It is important to note that it is not expected that the number of actual events of each species perfectly mirrors the distribution of these species. That is because the actual number of events might be affected by certain animals going through the beam  
20 several times and each species' physical distribution around and reaction to the laser beam. The fact that the classifier correctly predicts the trend of the mixed-species distributions shows that it does a good job of grouping the two species without *a priori* knowledge. The results also show that there is no immediate difference in whether the two species are attracted or repelled by the laser beam.  
25 Neither does it show any indication that the presence of another species in the water sample changes the expected fluorescence signal ratio. This is an important result as it indicates the feasibility of obtaining a library of expected fluorescence ratios for different species in single-species samples and using it for classification in a mixed-species sample.

30

In the above, an embodiment of a dual-wavelength-band fluorosensor has been described for the detection of zooplankton, in particular for detecting non-phytoplankton-eating zooplankton. The fluorosensor is configured to detect individual zooplankton organisms of 0.5 mm - 2mm body length, such as of 0.7  
35 mm - 1 mm body lengths.

The inventors have found that the sensitivity of the system depends on the gut content of the algae-eating species, but the system is robust even for starved algae-eaters. Based on measurements on single-species cultures of approximately 5 50 animals, an example of a classifier was developed that shows a high degree of discrimination, with a sensitivity of 98.9%. Finally, experiments have showed that the classifier is successful in grouping the two species in cultures of mixed species at different ratios.

10 While we have shown a good potential for discriminating salmon lice from algae-eating species, differentiation of salmon lice from other non-algae-eating species may further be facilitated by additionally considering other signatures than the dual-band fluorescence signal. Accordingly, some embodiments of the method and apparatus disclosed herein may further analyse the detected signals, e.g.  
15 captured images, so as to detect movement patterns and/or spatial features of the detected fluorescence patterns. The most abundant species around the Norwegian salmon farms are algae-eating. This is important since salmon lice abundances, in general, are low compared to the total abundance of zooplankton and high abundances of other non-algae-eating species may decrease the ability  
20 of the fluorosensor to detect variation in salmon lice numbers.

The apparatuses described herein may be used in a real-time monitoring system and may make use of machine learning to e.g. determine whether zooplankton is present within the volume of water and/or whether a zooplankton within the  
25 volume of water is a non-phytoplankton-eating zooplankton.

Some embodiments and aspects disclosed herein may be summarized as follows:

Embodiment 1: A method for detecting freely swimming non-phytoplankton-  
30 eating zooplankton, the method comprising:

- illuminating a volume of water with a first illumination radiation comprising a first illumination wavelength, the first illumination radiation being capable of inducing autofluorescence of chlorophyll;
- 35 - detecting radiation received from the volume of water;

- determining whether zooplankton is present within the volume of water based on the detected radiation;
  - 5 - measuring an amount of autofluorescence from chlorophyll from a zooplankton present within the volume of water;
  - identifying whether the zooplankton determined to be present within the volume of water is a non-phytoplankton-eating zooplankton based on the measured amount of autofluorescence from chlorophyll.
- 10

Embodiment 2: A method according to embodiment 1, wherein detecting radiation received from the volume of water comprises capturing an image of the volume of water and determining whether zooplankton is present within the volume of water  
15 comprises using a particle finding algorithm to locate zooplankton within the captured image.

Embodiment 3: A method according to any of the previous embodiments, wherein measuring an amount of autofluorescence from chlorophyll from a zooplankton  
20 present within the volume of water comprises measuring an intensity of fluorescent light from chlorophyll, the fluorescent light being received from the volume of water.

Embodiment 4: A method according to any of the previous embodiments, wherein  
25 detecting radiation received from the volume of water comprises recording blue autofluorescence from zooplankton within the volume of water and determining whether zooplankton is present within the volume of water is based on the recorded blue fluorescence.

30 Embodiment 5: A method according to embodiment 4, wherein the blue autofluorescence is measured in a wavelength range having end points within the range 400 - 650 nm, preferably within the range 450 - 600 nm, most preferably within the range 500 - 550 nm.

Embodiment 6: A method according to any of the previous embodiments, wherein the measured autofluorescence from chlorophyll is recorded in a wavelength range having end points within the range 650 - 750 nm, preferably within the range 650 - 725 nm, most preferably within the range 650 - 700 nm.

5

Embodiment 7: A method according to any of embodiments 4 - 6, wherein identifying whether the zooplankton is a non-phytoplankton-eating zooplankton comprises identifying non-phytoplankton-eating zooplankton from the amount of autofluorescence from chlorophyll and from the recorded blue autofluorescence.

10

Embodiment 8: A method according to any of embodiments 4 - 7, wherein identifying whether the zooplankton is a non-phytoplankton-eating zooplankton comprises:

- calculating a ratio of the measured amount of autofluorescence from chlorophyll from the zooplankton and a measured amount of the recorded blue autofluorescence from the zooplankton, and/or
- determining a first proportionality parameter for the measured amount of autofluorescence from chlorophyll from the zooplankton and determining a second proportionality parameter for a measured amount of the blue autofluorescence from the zooplankton, and

20

wherein identifying whether the zooplankton is a non-phytoplankton-eating zooplankton is further based on the calculated ratio and/or is further based on the determined proportionality parameters.

25 Embodiment 9: A method according to any of embodiments 1 - 3, wherein the method further comprises:

- illuminating the volume of water with a second illumination radiation comprising a second illumination wavelength;

wherein detecting radiation received from the volume of water comprises

30 recording an amount of reflected radiation from zooplankton within the volume of water, the reflected radiation comprising reflected radiation of the second illumination wavelength, and determining whether zooplankton is present within the volume of water is based on the recorded reflected radiation.

Embodiment 10: A method according to embodiment 9, wherein the first and second illumination radiation illuminate the volume of water at separate times.

Embodiment 11: A method according to any of embodiments 9 or 10, wherein the second illumination wavelength has a value within the wavelength range 650 - 750 nm, preferably within the range 650 - 725 nm, most preferably within the range 650 - 700 nm.

Embodiment 12: A method according to any of embodiments 9 - 11, wherein identifying whether the zooplankton is a non-phytoplankton-eating zooplankton comprises identifying non-phytoplankton-eating zooplankton from the amount of autofluorescence from chlorophyll and from the recorded reflected radiation.

Embodiment 13: A method according to any of embodiments 9 - 12, wherein identifying whether the zooplankton is a non-phytoplankton-eating zooplankton comprises calculating a ratio of the measured amount of autofluorescence from chlorophyll from the zooplankton and the recorded reflected radiation from the zooplankton.

Embodiment 14: A method according to any of the previous embodiments, the method further comprising reporting to a system or to a user whether one or more non-phytoplankton-eating zooplankton has been identified within the volume of water and/or an amount of non-phytoplankton-eating zooplankton having been identified within the volume of water.

25

Embodiment 15: A method according to any of the previous embodiments, comprising processing, by a processing unit, a captured image and/or other measurement results so as to detect one or more indicators of the presence of one or more individual organisms in the volume of water.

30

Embodiment 16: A method according to any of the previous embodiments, further comprising :

- determining where zooplankton are present within the volume of water, and

- measuring an amount of autofluorescence from chlorophyll and detecting fluorescent or reflected radiation from individual zooplankton.

Embodiment 17: An apparatus for detecting freely swimming non-phytoplankton-eating zooplankton, the apparatus comprising:

- a light source unit adapted to emit a first illumination radiation comprising a first illumination wavelength, the first illumination radiation being capable of inducing autofluorescence of chlorophyll;
- 10 - one or more optical elements configured to illuminate a volume of water with the first illumination radiation;
- a detector unit arranged to detect radiation from the volume of water;
- 15 - a processing unit configured to determine whether zooplankton is present within the volume of water based on the detected radiation;

wherein the detector unit is further configured to measure an amount of autofluorescence from chlorophyll from a zooplankton present within the volume  
20 of water, and the processing unit is further configured to determine whether a zooplankton within the volume of water is a non-phytoplankton-eating zooplankton based on the measured amount of autofluorescence from chlorophyll.

Embodiment 18: An apparatus according to embodiment 17, wherein the light  
25 source unit comprises a laser.

Embodiment 19: An apparatus according to any of embodiments 17 or 18, wherein the first illumination wavelength has a value within the range 365 nm - 740 nm, preferably within the range 365 - 650 nm.

30

Embodiment 20: An apparatus according to any of embodiments 17 - 19, wherein the detector unit is configured to record blue autofluorescence from zooplankton within the volume of water.

Embodiment 21: An apparatus according to embodiment 20, wherein the detector unit is configured to record blue autofluorescence in a wavelength range having end points within the range 400 - 650 nm, preferably within the range 450 - 600 nm, most preferably within the range 500 - 550 nm.

5

Embodiment 22: An apparatus according to any of embodiments 17 - 21, wherein the processing unit is further configured to:

- calculate a ratio of the measured amount of autofluorescence from chlorophyll from the zooplankton and a measured amount of the recorded blue autofluorescence from the zooplankton, and/or
- determine a first proportionality parameter for the measured amount of autofluorescence from chlorophyll from the zooplankton and determine a second proportionality parameter for the blue autofluorescence from the zooplankton, and

15 wherein the processing unit is further configured to determine whether the zooplankton is a non-phytoplankton-eating zooplankton based on the calculated ratio and/or based on the determined proportionality parameters.

Embodiment 23: An apparatus according to any of embodiments 17 or 18, 20 wherein:

- the light source unit is further configured to emit a second illumination radiation comprising a second illumination wavelength,
- the detector unit is further configured to record an amount of reflected radiation from zooplankton within the volume of water, the reflected radiation comprising reflected radiation of the second illumination wavelength, and
- the processing unit is further configured to determine whether zooplankton is present within the volume of water based on the recorded reflected radiation.

30

Embodiment 24: An apparatus according to embodiment 23, wherein the light source unit is further configured to illuminate the volume of water with the first and second illumination radiation at separate times.

Embodiment 24: An apparatus according to any of embodiments 23 - 24, wherein the second illumination wavelength has a value within the wavelength range 650 - 750 nm, preferably within the range 650 - 725 nm, most preferably within the range 650 - 700 nm.

5

Embodiment 26: An apparatus according to any of embodiments 23 - 25, wherein the detector unit comprises a detector and collection optics, the detector being placed in Scheimpflug angle with respect to the collection optics, the first and second illumination radiation being emitted by one or more light sources, the one or more light sources being co-aligned and the one or more light sources being arranged to lie in the Scheimpflug focal plane.

Embodiment 27: An apparatus according to any of embodiments 23 - 26, wherein the processing unit is further configured to:

- 15 - calculate a ratio of the measured amount of autofluorescence from chlorophyll from the zooplankton and the recorded reflected radiation from the zooplankton, and
- determine whether the zooplankton is a non-phytoplankton-eating zooplankton based on the calculated ratio.

20

Embodiment 28: An apparatus according to any of embodiments 17 - 27, wherein the measured autofluorescence from chlorophyll is recorded in a wavelength range having end points within the range 650 - 750 nm, preferably within the range 650 - 725 nm, most preferably within the range 650 - 700 nm.

25

Embodiment 29: A method according to any of embodiments 1 through 16, wherein the first illumination wavelength has a value within the range 365 nm - 740 nm, preferably within the range 365 - 650 nm.

### 30 LIST OF REFERENCE NUMBERS

- 10 Detector unit
- 12 Collection optics
- 14 Volume of water
- 15 Scheimpflug focal plane
- 35 16 Light source unit

18	Band pass filter
19	Processing unit
20	Field-of-view of the spectrometer tip
22	Red autofluorescence from chlorophyll
5 24	Blue autofluorescence from zooplankton
40	Fluorescence spectrum of non-phytoplankton-eating zooplankton
42	Fluorescence spectra of phytoplankton-eating zooplankton
60	Non-phytoplankton-eating zooplankton
62	Phytoplankton-eating zooplankton
10 80	Non-phytoplankton-eating zooplankton
82	Phytoplankton-eating zooplankton

## CLAIMS

1. A method for detecting freely swimming non-phytoplankton-eating zooplankton, the method comprising:
- 5 - illuminating a volume of water with a first illumination radiation comprising a first illumination wavelength, the first illumination radiation being capable of inducing autofluorescence of chlorophyll;
- detecting radiation received from the volume of water;
- 10 - determining whether zooplankton is present within the volume of water based on the detected radiation;
- measuring an amount of autofluorescence from chlorophyll from a zooplankton present within the volume of water;
- 15 - identifying whether the zooplankton determined to be present within the volume of water is a non-phytoplankton-eating zooplankton based on the measured amount of autofluorescence from chlorophyll.
- 20
2. A method according to claim 1, wherein detecting radiation received from the volume of water comprises capturing an image of the volume of water and determining whether zooplankton is present within the volume of water comprises using a particle finding algorithm to locate zooplankton within the captured image.
- 25
3. A method according to any of the previous claims, wherein measuring an amount of autofluorescence from chlorophyll from a zooplankton present within the volume of water comprises measuring an intensity of fluorescent light from chlorophyll, the fluorescent light being received from the volume of water.
- 30
4. A method according to any of the previous claims, wherein detecting radiation received from the volume of water comprises recording blue autofluorescence from zooplankton within the volume of water and determining whether zooplankton is present within the volume of water is based on the recorded blue
- 35 fluorescence.

5. A method according to claim 4, wherein the blue autofluorescence is measured in a wavelength range having end points within the range 400 - 650 nm, preferably within the range 450 - 600 nm, most preferably within the range 500 - 550 nm.
6. A method according to any of the previous claims, wherein the measured autofluorescence from chlorophyll is recorded in a wavelength range having end points within the range 650 - 750 nm, preferably within the range 650 - 725 nm, most preferably within the range 650 - 700 nm.
7. A method according to any of claims 4 - 6, wherein identifying whether the zooplankton is a non-phytoplankton-eating zooplankton comprises identifying non-phytoplankton-eating zooplankton from the amount of autofluorescence from chlorophyll and from the recorded blue autofluorescence.
8. A method according to any of claims 4 - 7, wherein identifying whether the zooplankton is a non-phytoplankton-eating zooplankton comprises:
- calculating a ratio of the measured amount of autofluorescence from chlorophyll from the zooplankton and a measured amount of the recorded blue autofluorescence from the zooplankton, and/or
  - determining a first proportionality parameter for the measured amount of autofluorescence from chlorophyll from the zooplankton and determining a second proportionality parameter for a measured amount of the blue autofluorescence from the zooplankton, and
- wherein identifying whether the zooplankton is a non-phytoplankton-eating zooplankton is further based on the calculated ratio and/or is further based on the determined proportionality parameters.
9. A method according to any of claims 1 - 3, wherein the method further comprises:
- illuminating the volume of water with a second illumination radiation comprising a second illumination wavelength;
- wherein detecting radiation received from the volume of water comprises recording an amount of reflected radiation from zooplankton within the volume of

water, the reflected radiation comprising reflected radiation of the second illumination wavelength, and determining whether zooplankton is present within the volume of water is based on the recorded reflected radiation.

5 10. A method according to claim 9, wherein the first and second illumination radiation illuminate the volume of water at separate times.

11. A method according to any of claims 9 or 10, wherein the second illumination wavelength has a value within the wavelength range 650 - 750 nm, preferably  
10 within the range 650 - 725 nm, most preferably within the range 650 - 700 nm.

12. A method according to any of claims 9 - 11, wherein identifying whether the zooplankton is a non-phytoplankton-eating zooplankton comprises identifying non-phytoplankton-eating zooplankton from the amount of autofluorescence from  
15 chlorophyll and from the recorded reflected radiation.

13. A method according to any of claims 9 - 12, wherein identifying whether the zooplankton is a non-phytoplankton-eating zooplankton comprises calculating a ratio of the measured amount of autofluorescence from chlorophyll from the  
20 zooplankton and the recorded reflected radiation from the zooplankton.

14. A method according to any of the previous claims, the method further comprising reporting to a system or to a user whether one or more non-phytoplankton-eating zooplankton has been identified within the volume of water  
25 water and/or an amount of non-phytoplankton-eating zooplankton having been identified within the volume of water.

15. A method according to any of the previous claims, comprising processing, by a processing unit, a captured image and/or other measurement results so as to  
30 detect one or more indicators of the presence of one or more individual organisms in the volume of water.

16. A method according to any of the previous claims, further comprising:  
- determining where zooplankton are present within the volume of water, and

- measuring an amount of autofluorescence from chlorophyll and detecting fluorescent or reflected radiation from individual zooplankton.

17. An apparatus for detecting freely swimming non-phytoplankton-eating  
5 zooplankton, the apparatus comprising:

- a light source unit adapted to emit a first illumination radiation comprising a first illumination wavelength, the first illumination radiation being capable of inducing autofluorescence of chlorophyll;
- 10 - one or more optical elements configured to illuminate a volume of water with the first illumination radiation;
- a detector unit arranged to detect radiation from the volume of water;
- 15 - a processing unit configured to determine whether zooplankton is present within the volume of water based on the detected radiation;

wherein the detector unit is further configured to measure an amount of autofluorescence from chlorophyll from a zooplankton present within the volume  
20 of water, and the processing unit is further configured to determine whether a zooplankton within the volume of water is a non-phytoplankton-eating zooplankton based on the measured amount of autofluorescence from chlorophyll.

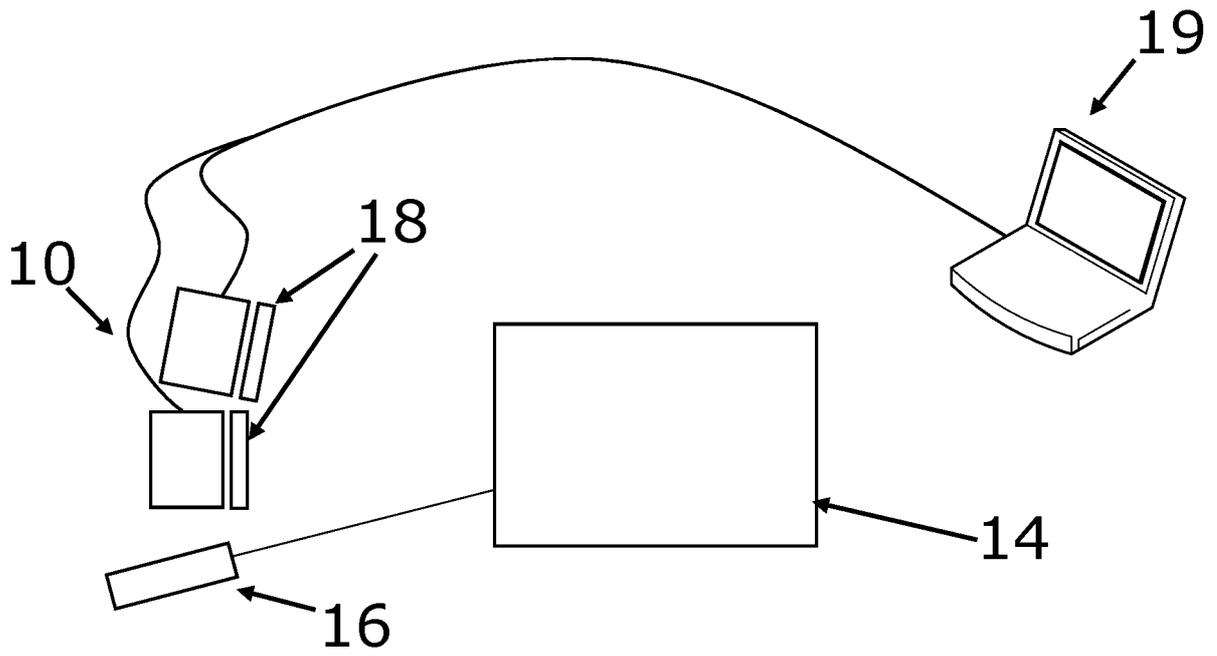


FIG. 1A

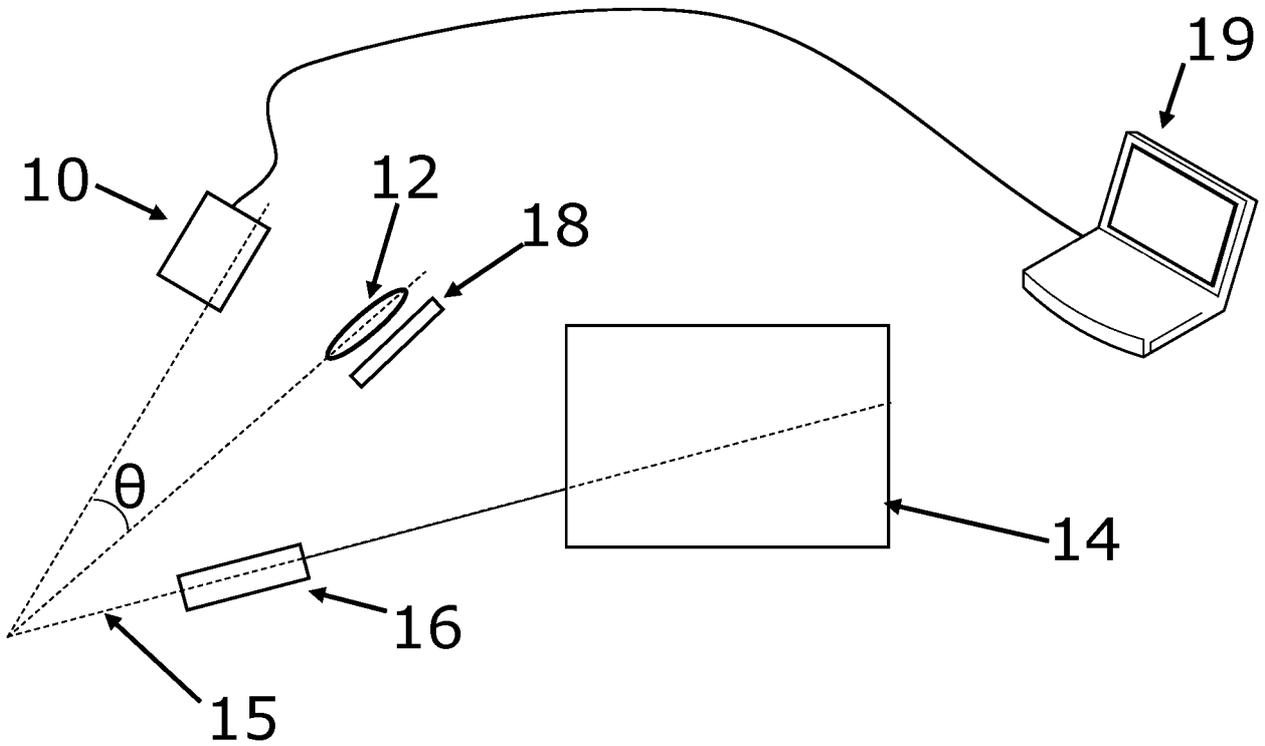


FIG. 1B

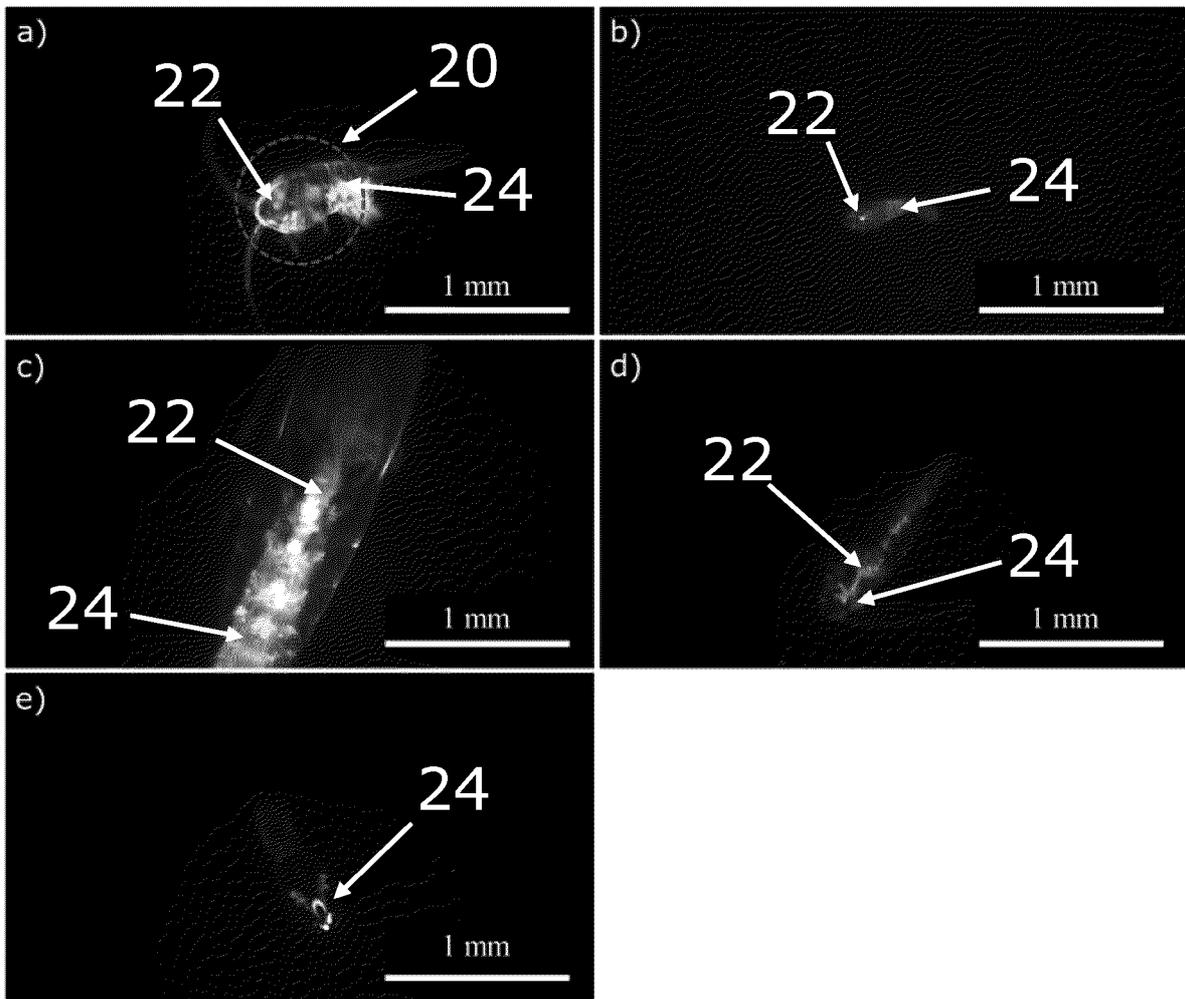


FIG. 2

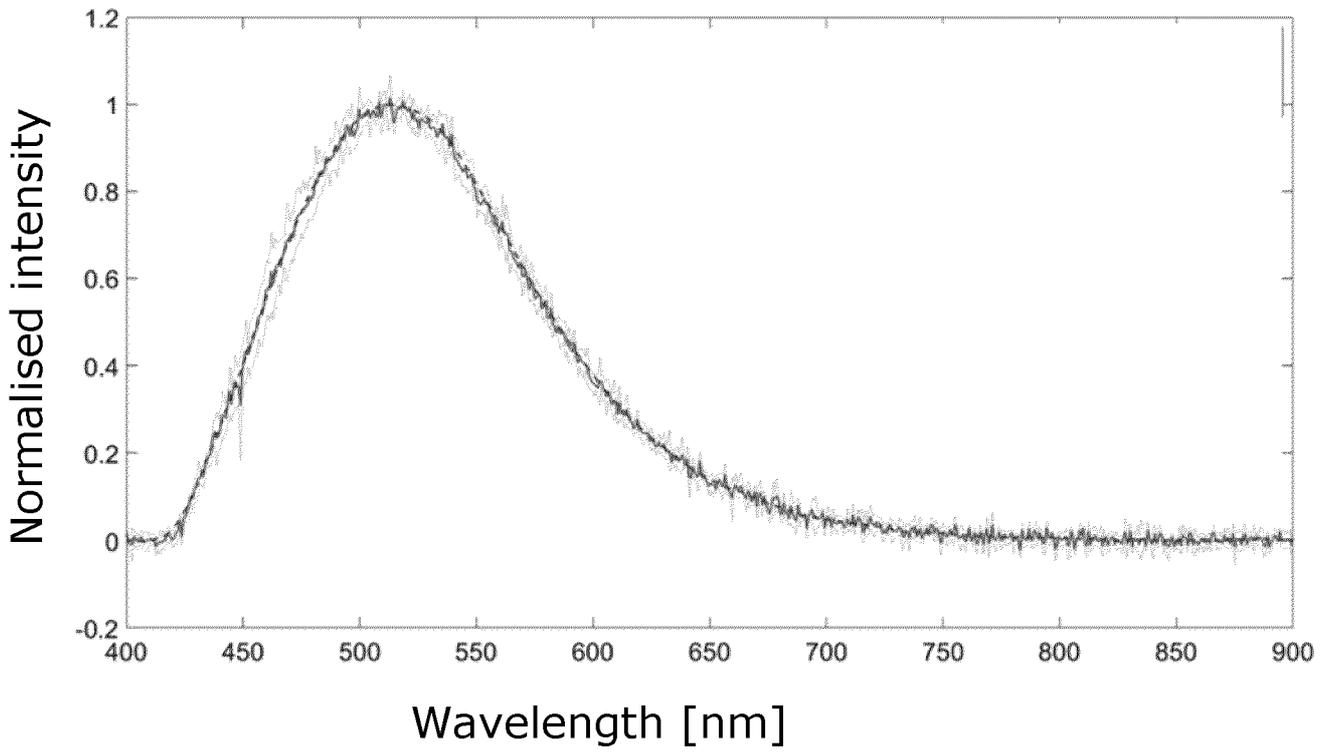


FIG. 3

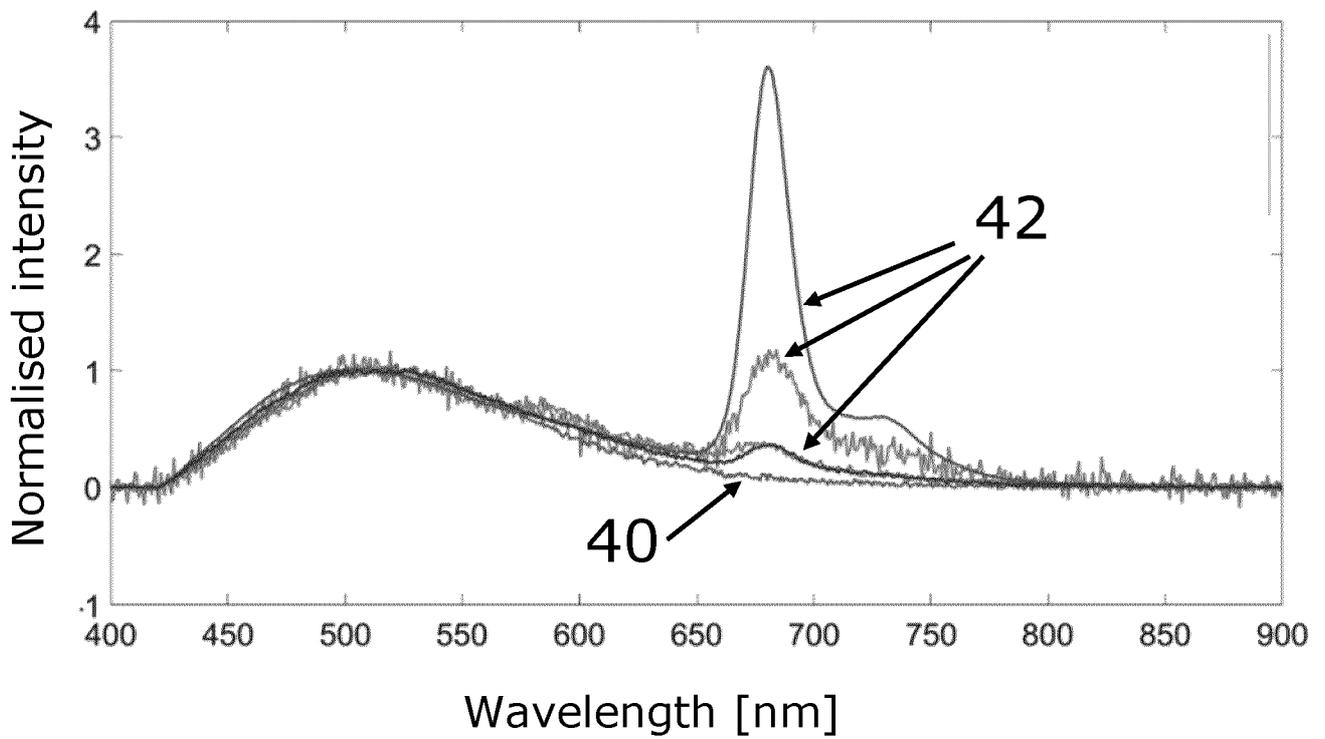


FIG. 4

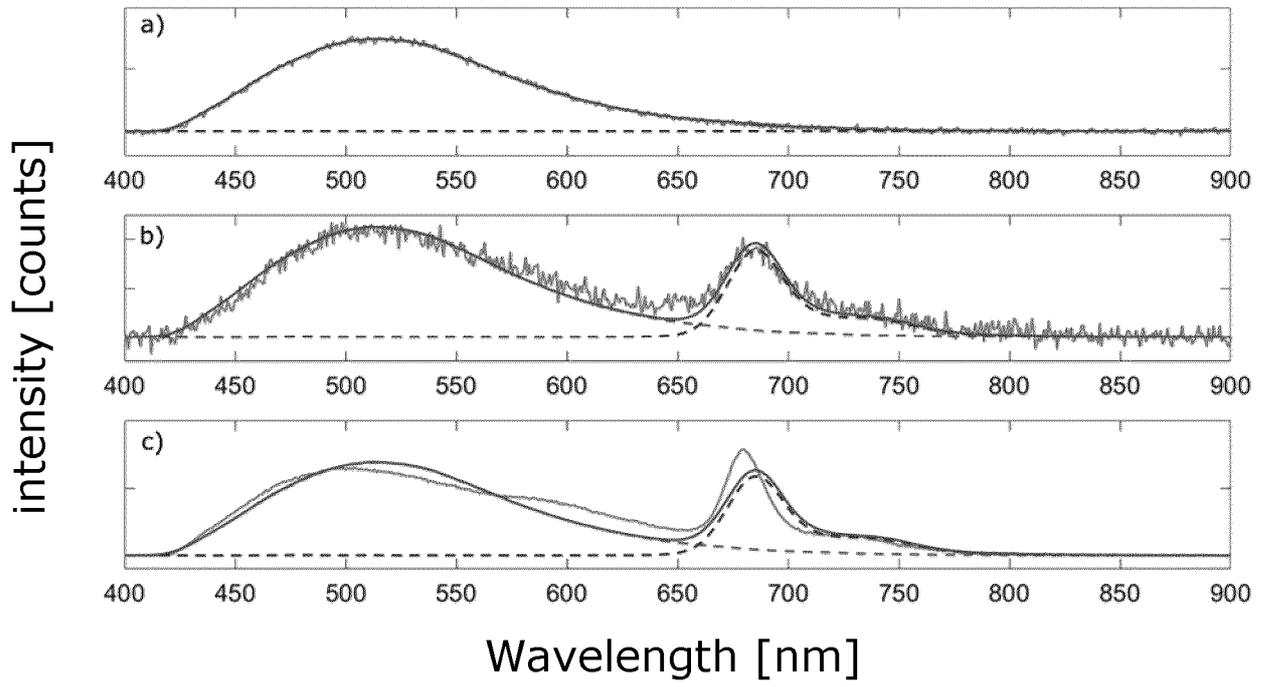


FIG. 5

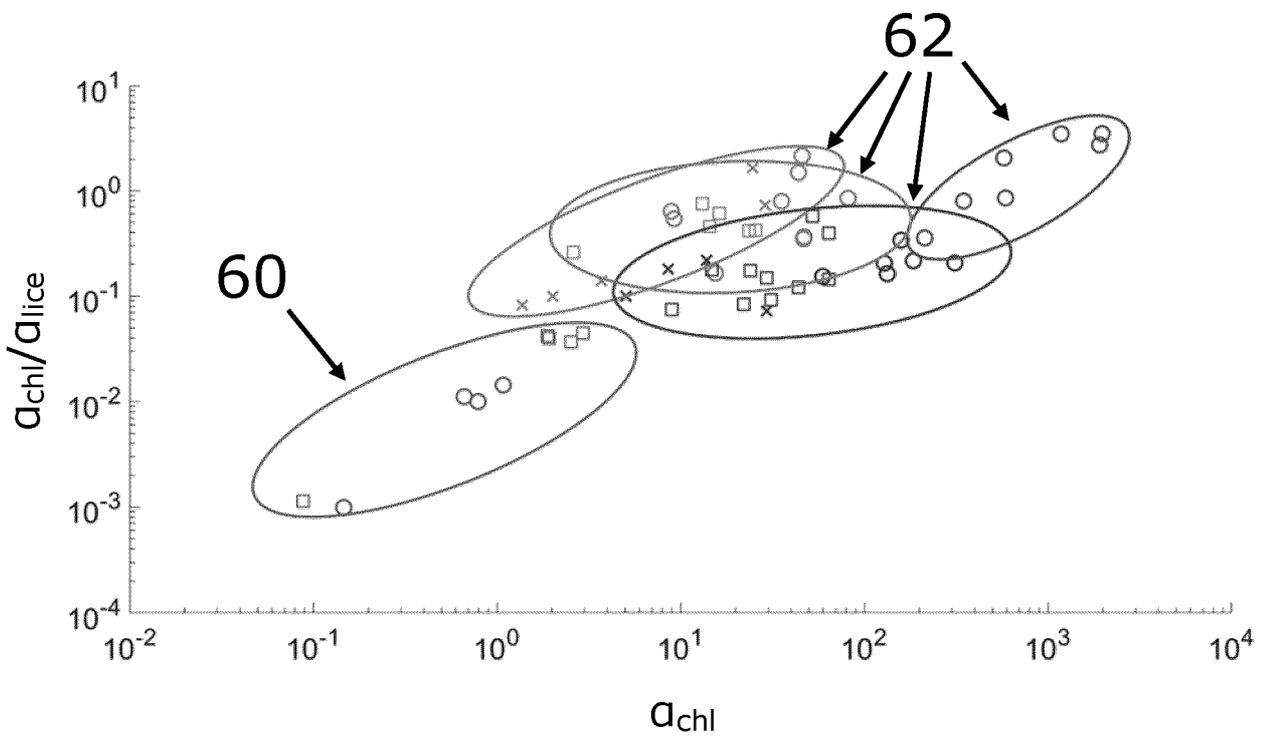


FIG. 6

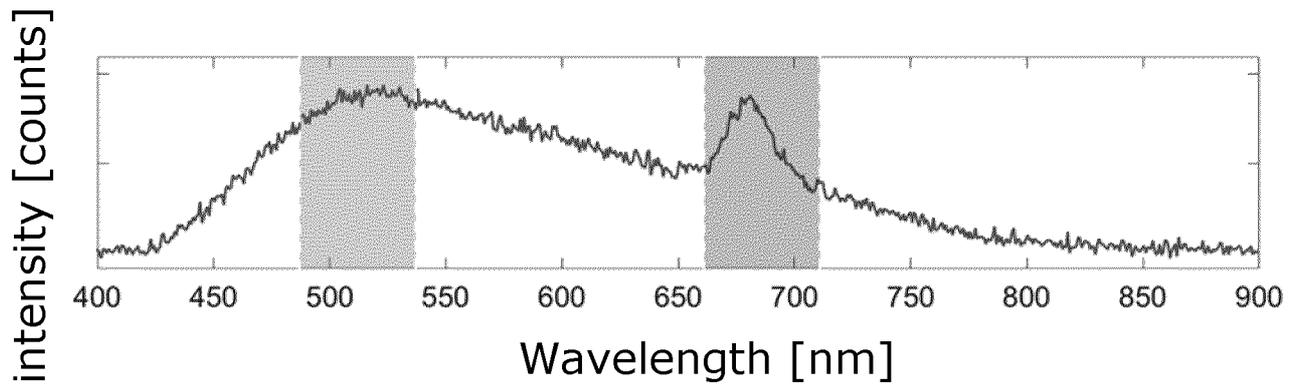


FIG. 7

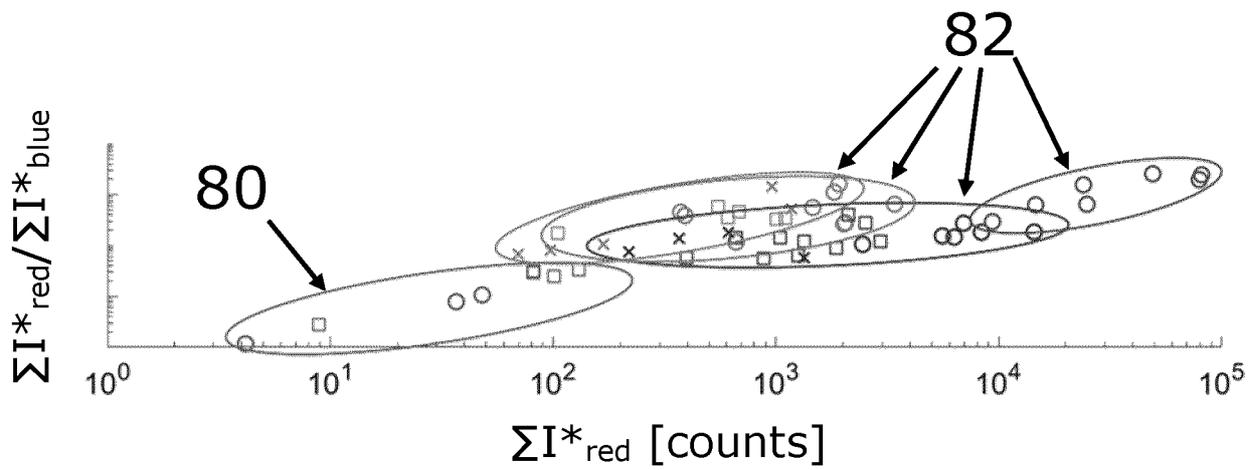


FIG. 8

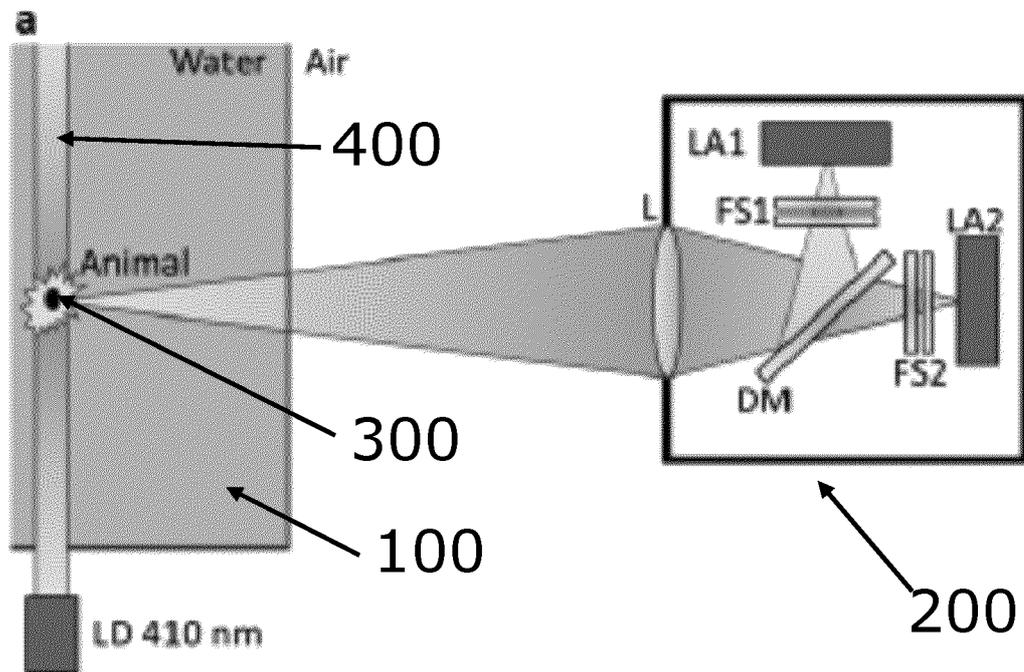


FIG. 9

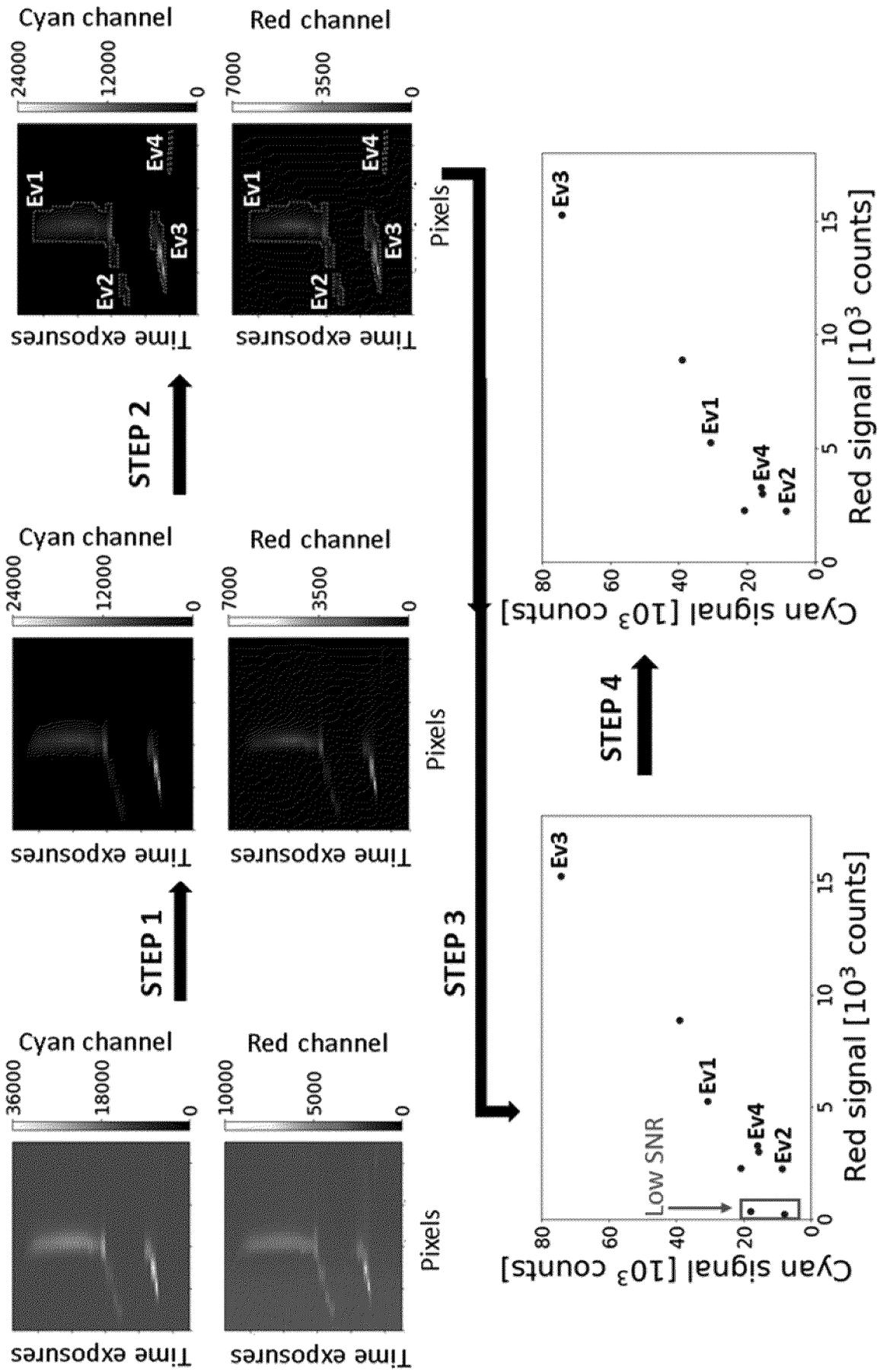


FIG. 10

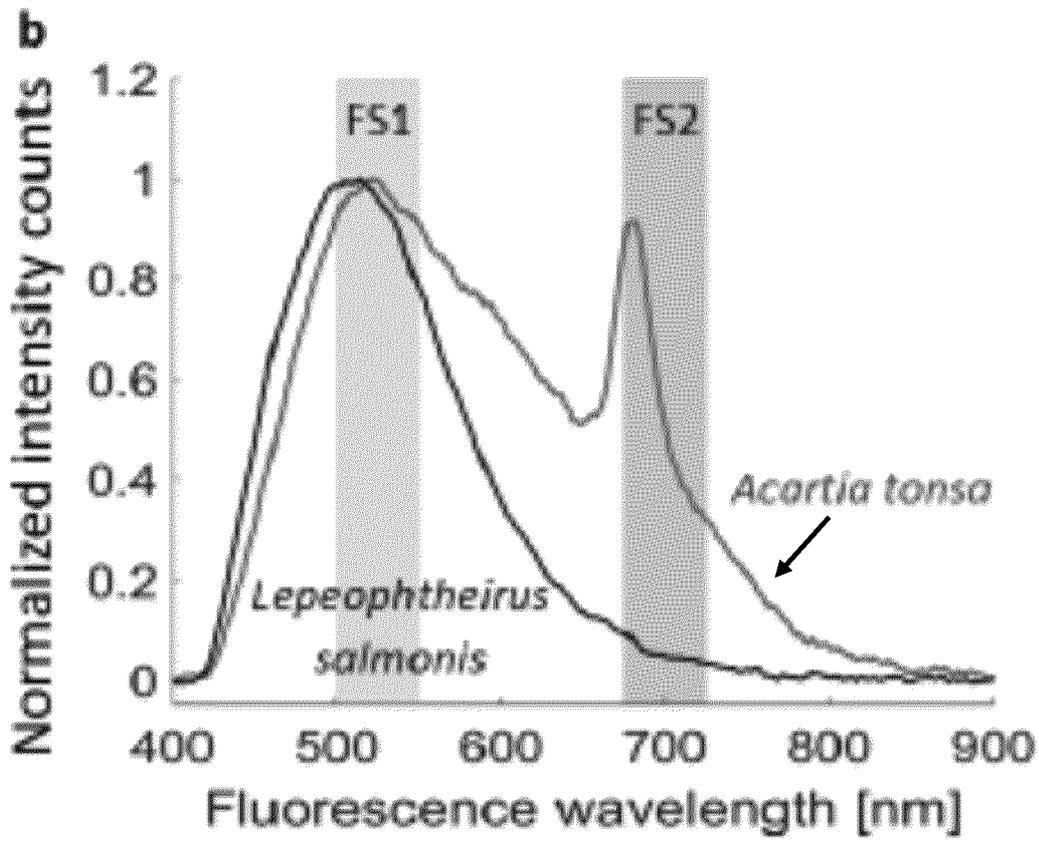


FIG. 11

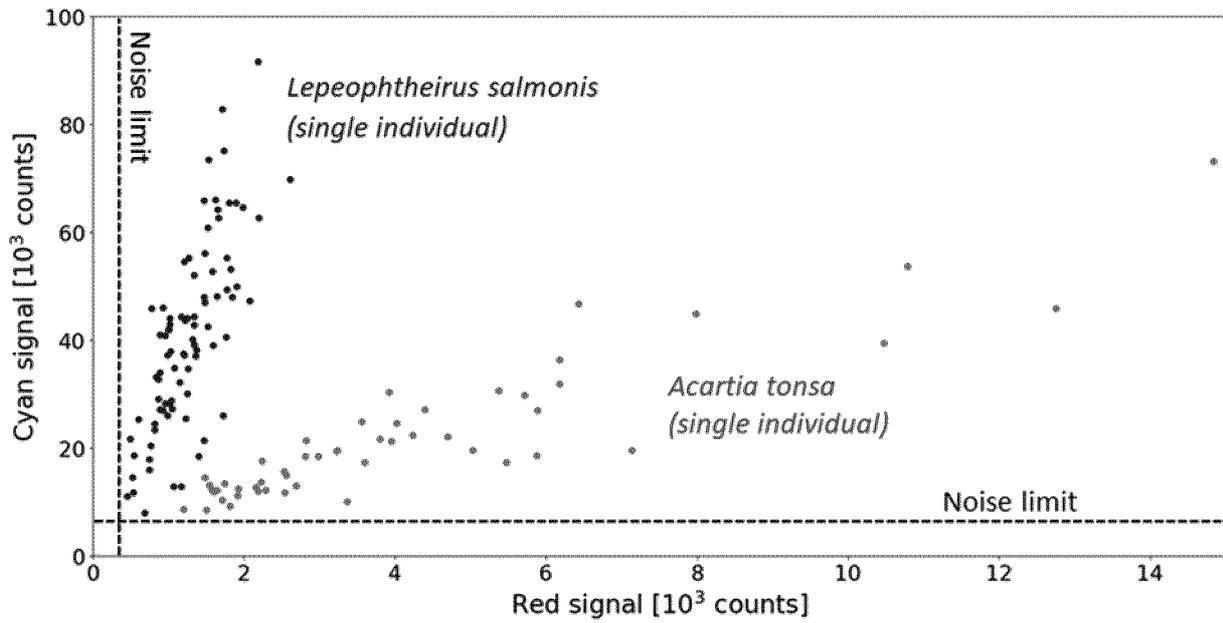


FIG. 12

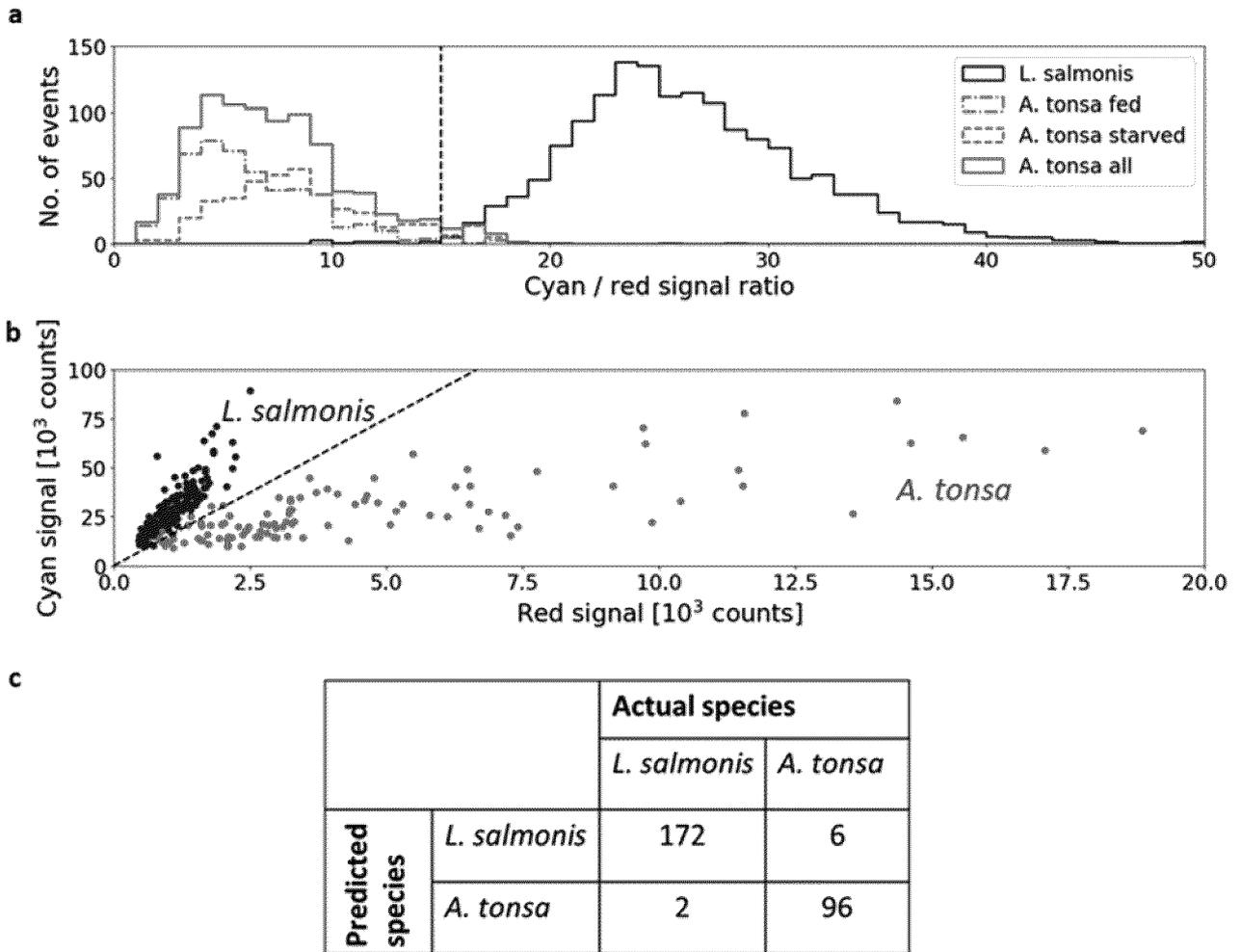


FIG. 13

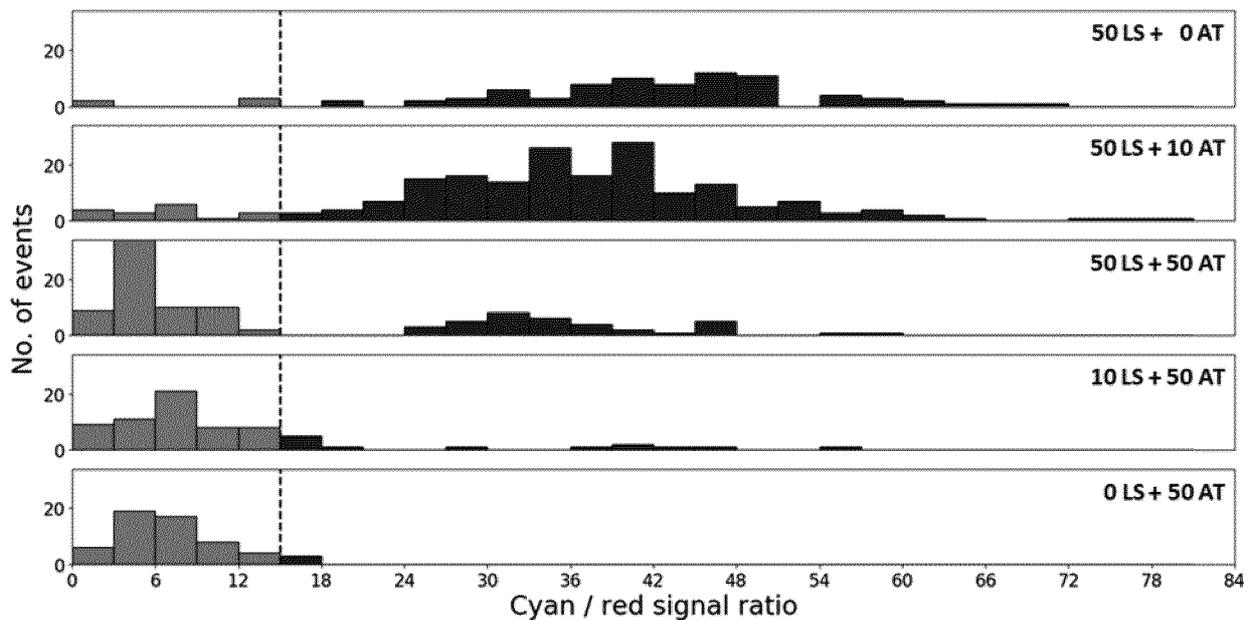


FIG. 14

**INTERNATIONAL SEARCH REPORT**

International application No  
**PCT/EP2020/064696**

A. CLASSIFICATION OF SUBJECT MATTER  
**INV. G01N2 1/64**  
**ADD.**

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)  
**G01N**

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)  
**EPO - Interna l , WPI Data , INSPEC**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2018/105414 A1 (SATAKE ENG CO LTD [JP]) 14 June 2018 (2018-06-14)	1-6,9-17
A	abstract paragraphs [0011] - [0021], [0038] - [0065]	7,8
Y	H. COELHO ET AL: "Nondestructive quantification of phytoplankton gut content of brachyuran crab megalopae using in vivo chlorophyll a fluorescence", JOURNAL OF PLANKTON RESEARCH, vol. 31, no. 5, 15 January 2009 (2009-01-15), pages 577-581, XP055636807, Oxford University Press ISSN: 0142-7873, DOI: 10.1093/plankt/fbp009 page 577 - page 579	1-6,9-17
A	----- -/--	7,8

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
- "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 <b>28 July 2020</b>	Date of mailing of the international search report <b>05/08/2020</b>
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer <b>Meacher, David</b>

## INTERNATIONAL SEARCH REPORT

International application No  
PCT/EP2020/064696

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Erdem M Karaköylü ET AL: "Copepod feeding quantified by planar laser imaging of gut fluorescence", Limnology and Oceanography: Methods, 1 January 2009 (2009-01-01), pages 33-41, XP055636814, DOI: 10.4319/lom.2009.7.33 Retrieved from the Internet: URL:https://aslopubs.onlinelibrary.wiley.com/doi/abs/10.4319/lom.2009.7.33	1-6,9-17
A	abstract	7,8
Y	----- C. S. DAVIS ET AL: "The Video Plankton Recorder (VPR)", ARCHIV FUR HYDROBIOLOGIE, BEIHEFTE, ERGEBNISSE DER LIMNOLOGIE, vol. 36, 1 July 1992 (1992-07-01), pages 67-81, XP055121598, Stuttgart abstract; figure 1	2,15,16
Y	----- TSAI AN YI ET AL: "The effect of grazing and viral lysis on the diel variations of Synechococcuspp. abundance in the East China Sea", ESTUARINE, COASTAL AND SHELF SCIENCE, NEW YORK, NY, US, vol. 163, 24 June 2015 (2015-06-24), pages 108-115, XP029280928, ISSN: 0272-7714, DOI: 10.1016/J.ECSS.2015.06.020 page 111, left-hand column, lines 1-5	4,5
Y	----- US 2009/093045 A1 (TAKENAKA KEI [JP] ET AL) 9 April 2009 (2009-04-09) figure 4	15
A	----- EP 2 962 556 A1 (ARDEO TECHNOLOGY AS [NO]) 6 January 2016 (2016-01-06) cited in the application abstract	1-17
A	----- JP 2010 063403 A (IHI CORP) 25 March 2010 (2010-03-25) paragraphs [0018] - [0019] ----- -/--	1-17

## INTERNATIONAL SEARCH REPORT

International application No  
PCT/EP2020/064696

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>B. A. HENSE ET AL: "Use of fluorescence information for automated phytoplankton investigation by image analysis", JOURNAL OF PLANKTON RESEARCH, vol. 30, no. 5, 11 February 2008 (2008-02-11), pages 587-606, XP055636745, Oxford University Press ISSN: 0142-7873, DOI: 10.1093/plankt/fbn024 abstract</p> <p style="text-align: center;">-----</p>	1-17

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No <b>PCT/EP2020/064696</b>
--

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
<b>wo 2018105414</b>	<b>A1</b>	<b>14-06-2018</b>	
		<b>AU 2017372183</b>	<b>A1 04-07-2019</b>
		<b>CN 110062805</b>	<b>A 26-07-2019</b>
		<b>EP 3553164</b>	<b>A1 16-10-2019</b>
		<b>JP 2018093758</b>	<b>A 21-06-2018</b>
		<b>KR 20190094189</b>	<b>A 12-08-2019</b>
		<b>TW 201821616</b>	<b>A 16-06-2018</b>
		<b>US 2020087611</b>	<b>A1 19-03-2020</b>
		<b>wo 2018105414</b>	<b>A1 14-06-2018</b>
-----			
<b>US 2009093045</b>	<b>A1</b>	<b>09-04-2009</b>	
		<b>JP 2009085898</b>	<b>A 23-04-2009</b>
		<b>US 2009093045</b>	<b>A1 09-04-2009</b>
-----			
<b>EP 2962556</b>	<b>A1</b>	<b>06-01-2016</b>	<b>NONE</b>
-----			
<b>JP 2010063403</b>	<b>A</b>	<b>25-03-2010</b>	<b>NONE</b>
-----			