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Research Article

Development a Monitoring System for Detecting Marine Microorganisms in Ballast Water

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Abstract

The problems of marine invasive species carried by ballast water are greatly severe due to the growing trade using shipping, especially last two decades. Ballast water treatment systems to tackle the problems have been developed. However we need a monitoring system to evaluate the performance of the treatment systems. In this study, a monitoring system using micro fluidic devices has been developed to detect living marine species in ballast water. The monitoring system consists of two sub-systems; one is for 10-50 μm species and the other for >50 μm ones, which was designed to be used in situ on ships. The results of this study showed that the developed system to assess the working performance of the ballast water treatment system will be useful in the international commercial ships.

Keywords: Ballast water; Marine bio pollution; Monitoring system; Phytoplankton; Zoo plankton

Introduction

Ballast water is used essentially to maintain stability and safety of ships during no shipping [1-2]. Ballast water is pumped-in to maintain safe operating conditions throughout a voyage. This practice reduces stress on the hull, provides transverse stability, improves propulsion and maneuverability, and compensates for weight lost due to fuel and water consumption. Since all ships are designed for a certain weight range, ballast is used to compensate forum loaded cargo [3]. International Maritime Organization (IMO) estimates that each year about 10 billion tons of ballast water are transported and exchanged around the world during maritime shipping [4]. However the problems of marine invasive species carried by ballast water are greatly severe due to the growing trade using shipping, especially last decade.

Furthermore some non-invasive species may become invasive and negatively affect native species or near shore habitats. So the treatment, sampling and assess men of the marine species in the ballast water and marine environments have been paid extensive attention throughout the globe [5-21]. Although there are so many researches on the ballast water treatment systems [2, 6, 12, 14-16, 20, 22], the monitoring system to assess the treatment system has been rarely reported to date.

In this study, we developed a monitoring system to detect living marine species in ballast water. The monitoring system consists of two sub-systems; one is for 10~50 μm species and the other for >50 μm ones, which was designed to be used *in situ* on ships. The fluorescence due to the chlorophyll and the dyes is used for detecting the 10-50 μm species while the movement of organisms is used for detecting the >50 μm species. The results of this study showed that the developed system to assess the working performance of the ballast water treatment system will be useful in the international commercial ships.

Experiments

Image analysis with time: for >50 μm species

For the relatively large species (i.e. >50 μm in this study), the motion of the organism was monitored to detect alive ones. The bio species, which are >50 μm, are easily observed using ×20 lens. Figure 1 shows the counting chamber (a) and the counting point (b). The counting chamber is made using PDMS to obtain CCD images. The chamber is evacuated, and then is filled with 10 ml of concentrated sample. Using CCS camera, we simply obtained the images of the sample with 30 sec of time period at the indicated five regions in (Figure 1) (b). (Figure 2) shows the image analysis process; (a) taking an original CCD image, (b) converting into binary image, and (c) selecting an object. The whole process is as follows;

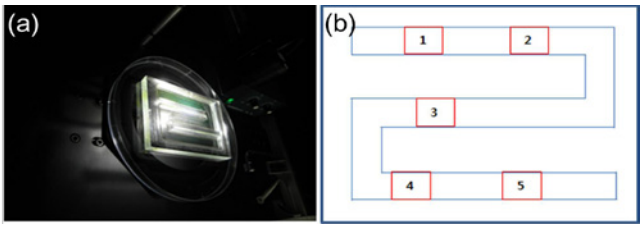

Figure 1: The counting chamber; (a) a real picture and (b) the counting regions.

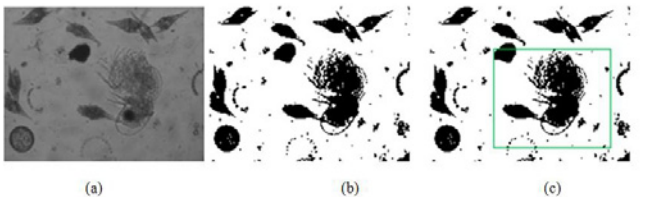

Figure 2: Image analysis process for >50 μm species. (a) Original CCD image (b) Converting into binary image (c) Selecting an object.

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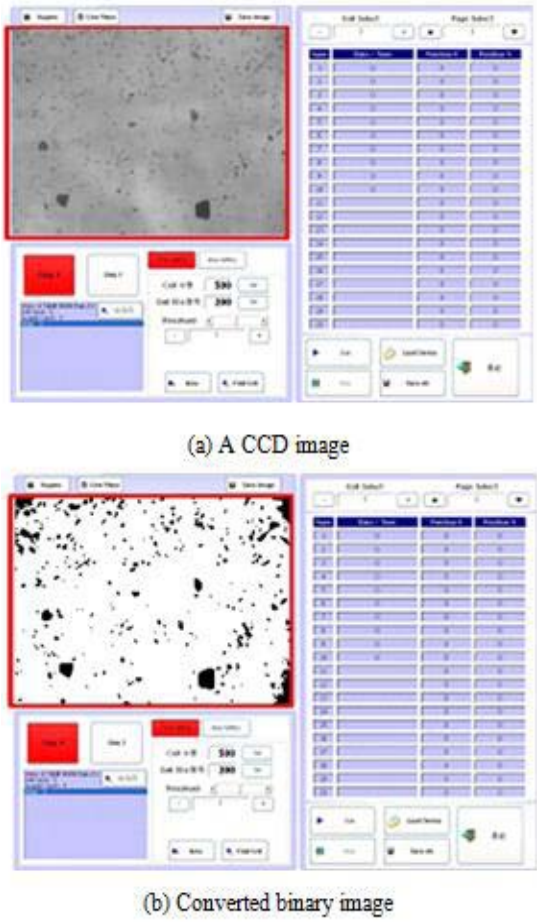

Figure 3: Screen shots of the counting system developed for >50 μm species.

Take an image using CCD camera;

Convert the CCD image into binary one;

Select individual objects and record their shapes;

Give a number to each object;

Take an CCD image after 30 sec and analyze the movement of each object, and then;

Count the moved objects which are regarded as living organisms.

We have developed a system using which the process described above is carried automatically out. (Figure 3) shows the screen shots of the counting system developed for >50 μm species.

Image analysis using fluorescence: for 10~50 μm specie

Some microorganisms can emit fluorescence by absorbing specific light source (i.e. self-fluorescence). For example, the chlorophyll in phytoplankton emits red-fluorescence by absorbing blue light. The emitting light intensity reflects the viability of the plankton, in turns there is no emission if the plankton is dead. On the other hand, the others are not able to emit fluorescence themselves even though they are alive. For these microorganisms, the Fluorescein Di Acetate (FDA) and Calcein-AM were used to stain them. The fluorescence dyes interacts with the esterase, esterase and lipases in the living organisms, consequently emitting high-intensity green-fluorescence (>515 nm wavelength) by absorbing blue light (440-490 nm wavelength).

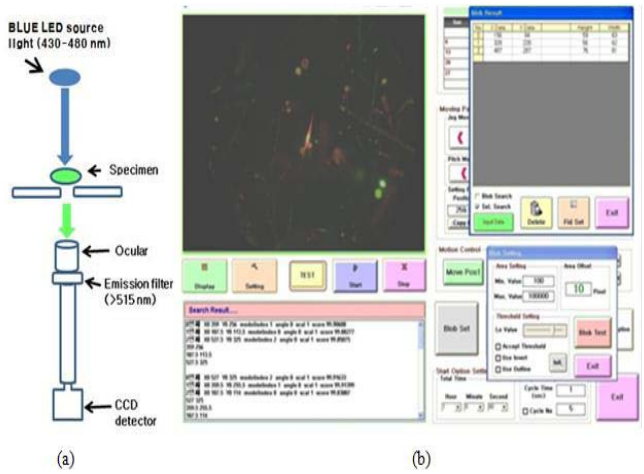

Figure 4: The counting system developed for 10~50 μm species. (a) Schematic of the counting apparatus (b) Screen shot of the counting system.

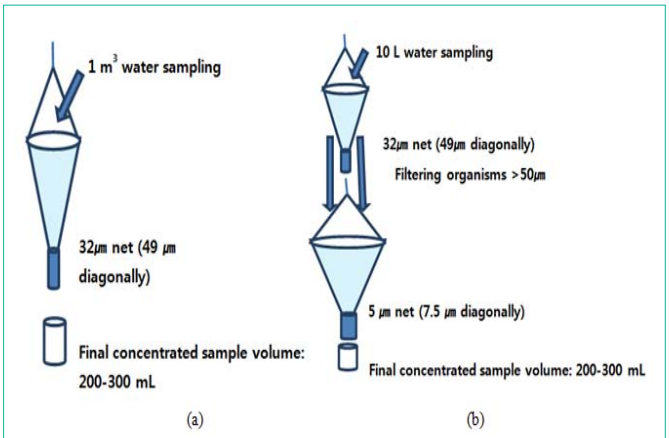

Figure 5: Schematics of the sample concentration systems; (a) for >50 μm species and (b) for 10~50 μm ones.

Both the self-fluorescent organisms and the stained ones absorb blue light and then emit red- and green-fluorescence, respectively. Soa blue-LED was used to excite the chlorophyll and the dyes in the target organisms .And a >515 nm emission filter was used to recognize the red-fluorescence and the green-fluorescence due to the chlorophyll and the dyes in the living organisms, respectively. (Figure 4) shows the schematic of the counting system developed for 10-50 μm species. After the setup of the apparatus (Figure 4a), the counting process was carried automatically out using the system developed in this study as shown in (Figure 4b).

Testing the counting systems

The water samples were collected at the Masan Port, the Seomjin-gang estuary, and the Sapgyo-ho around the Korean Peninsula. And then the collected samples were concentrated using the filtering nets. For >50 μm-sized species, a net with 32-μm mesh (the diagonal is ~49.9 μm) was chosen while another one with 5-μm mesh (the diagonal is ~7.5 μm) was chosen for 10~50 μm-sized species. 1,000 *l* of

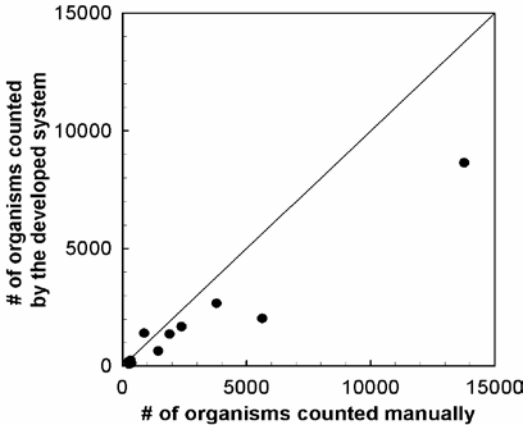

Figure 6: The total number of >50 μm species in 10^6 ml sample; estimated by the developed system *vs.* counted manually.

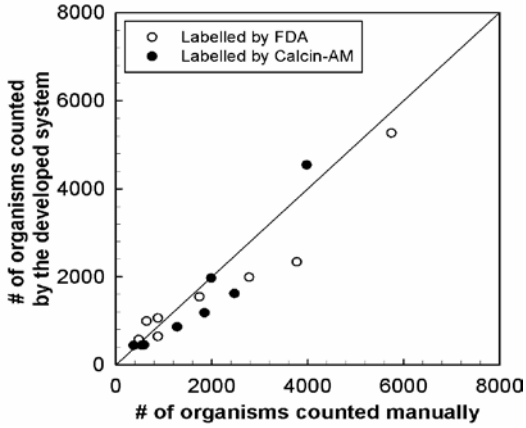

Figure 7: The total number of 10-50 μm species in 1 ml sample; estimated by the developed system vs. counted manually.

the collected water were used for concentrating >50 μm-sized species, consequently obtained 200-300 ml of the concentrated sample, while 10 *l* of that was used for concentrating 10~50 μm-sized species to obtain 200-300 ml of the concentrated sample. The schematics of the concentration systems are depicted in (Figure 5).

Results and Discussion

Counting the >50 μm organisms

The counted number of organisms, using the system as shown in Figure 1-3, was used to estimate the total number of it. The total number of the organisms in 10^6ml sample N_{tot} could be estimated by following equation;

$$N_{tot} = a \times (b/c) \times d \quad (1)$$

Where a is the counted number of the organisms in the counting regions, b (=24.5 cm^2) is the area of the counting chamber, c (=6.5×5 cm^2) is the sum area of the counting regions and d (=10^6 ml/ 10 ml) is the constant for restoring into the total volume.

(Figure 6) shows the total number of >50 μm species in 10^6 ml sample; counted by the developed system vs. counted manually. The numbers of the organisms counted by the system are lower than those by manual counting except just one case. The counting

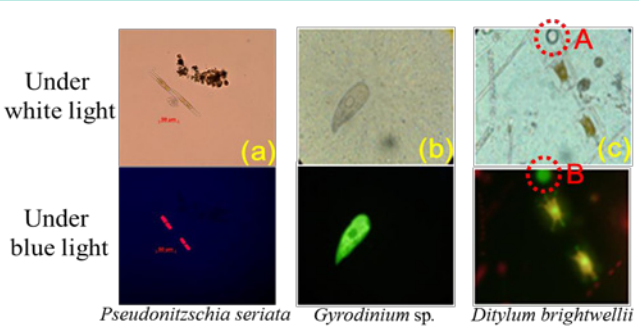

Figure 8: Images of the tested organisms under white (upper) and blue (bottom) lights; (a) *Pseudonitzschia seriata* (self-fluorescence), (b) *Gyrodinium* sp. (labeled by FDA) and (c) *Ditylum brightwellii* (labeled by Calcein-AM).

system's deviations from the manual counting are less than 67 %. The counting errors are attributed that some organisms are being a cluster because they are not perfectly separated from each other. Also some organisms did not move during the measurement because they have lost their activity.

Counting the 10~50 μm organisms

The concentrated 10-50 μm organisms were labeled by FDA and Calcein-AM and then the living organisms were counted by the developed counting systems as shown in (Figure 4b). (Figure 7) shows the total number of 10-50 μm organisms in 1 ml sample; counted by the developed system vs. counted manually. The counted numbers of the organisms using the developed system are in good agreement with those of the manual counting .The counting system's deviations from the manual

Counting is in range of -22%~38% except just one case. For some cases the organism numbers manually counted are less than those by the counting system contrasted to the cases of the >50 μm, which is attributed due to the cluster of excessive dyes and/or the labeled dust in the sample liquid. Figure 5 shows some typical results of the present study, images of (a) *Pseudonitzschia seriata* (self-fluorescence), (b) *Gyrodinium* sp. (labeled by FDA) and (c) *Ditylum brightwellii* (labeled by Calcein-AM). As shown in (Figure 5c), there is a droplet which is not an organism (A, the dotted circle at upper image), however the droplet is emitting green light under blue light as shown in the B (the dotted circle at bottom image). This can explain why the counting system's errors have been encountered.

Discussion about the developed counting systems for the organisms in ballast water

There are so many species in ballast water. Therefore the organisms should be separated each other with reference to their size and/or characteristics to counter living one. Most zooplankton are larger than 50 μm and they can move themselves, while most phytoplankton are less than 50 μm and they can emit fluorescence themselves or due to the labeled dyes.

For the zooplankton, most case undercounted the living organisms as shown in (Figure 6), which is attributed to the low-activity of some of them. On the other hand, for the phytoplankton the numbers of living one were over-counted as shown in (Figure 7), which is speculated due to the dust and the excess dyes as shown in (Figure 8).

Conclusion

Ballast water system is essential for international commercial ships. However the problems of marine invasive species carried by the ballast water are greatly severe due to the growing trade using shipping, especially last two decades. The monitoring system to assess the ballast water treatment system has been rarely reported to date although numerous researches on the treatment method have been carried out. We developed a system to counter living organisms in the ballast water. The system consists of two sub-systems; one is for 10-50 μm species and the other for >50 μm ones, which was designed to be used *in situ* on ships. The results of the present study showed that the developed system to count living organisms in the ballast water will be useful in the international commercial ships.

Acknowledgement

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