BIORESEARCH BULLETIN
Sapience Research Labs

ORIGINAL PAPER OPEN ACCESS

Observation of phytoplankton cells using a small hand-held microscope with an LED lamp for field surveys

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Received: 05 June 2014 / **Accepted:** 01 July 2014 / **Published online:** 11 October 2014

Abstract

The use of a small hand-held microscope with a battery-powered LED lamp for field surveys of phytoplankton was investigated. Although it was difficult to observe the details of phytoplankton cells, this type of microscope is useful in field surveys because it is convenient for quickly examining a collected sample.

Keywords: phytoplankton, monitoring, sampling, hand-held microscope

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Web address:
http://bioresonline.org/archives/A185.pdf

Article citation:
Tatsuya Yurimoto, Faizul Mohd Kassim and Alias Man. Observation of phytoplankton cells using a small hand-held microscope with an LED lamp for field surveys**.** Bioresearch Bulletin (2014) 2: 01-05.

INTRODUCTION

Environmental monitoring of fishing grounds requires rapid and detailed surveying depending on the occurrence of phytoplankton species (Li and Smayda, 2000). In particular, harmful or toxic species can damage fishing industries (Anderson *et al.*, 2000; Hoagland *et al.*, 2002); thus delayed surveying can lead to serious economic losses. In addition, harmful and toxic species including Raphidophyceae and unarmored dinoflagellates cells are difficult to preserve by formalin fixation (Li and Smayda, 2000; Matsuoka *et al.*, 2004; Hiroishi *et al.*, 2005). Therefore, phytoplankton samples should ideally be observed immediately after sampling. Samples collected by phytoplankton nets or water bottles in a survey area are normally returned to a laboratory, where they are analyzed to identify and count cells. Thus, a given plankton species contained in the collected sample can only be confirmed by a detailed survey performed the day after sampling or later. However, if it is possible to observe a phytoplankton species in the field, the occurrence of the species could be confirmed immediately. Thus, the decision to carry out a detailed survey can be made immediately on the basis of the results. However, a normal light microscope is large and requires a power source, making it impractical for use in the field. Accordingly, portable outdoor microscopes are utilized during Japanese phyto-plankton research, including the CMS400 (Carton Co. Ltd., Tokyo, Japan); one of the current authors has used this model for field observations. This microscope is designed to be portable for outdoor use and has sufficient magnification for observing phytoplankton. However, it is expensive and priced same as a normal light microscope and requires an attached light source using a battery-powered tungsten lamp.

Many small microscopes with battery-powered LED lamps have recently become available. Surveys of related models, which have 40–100× magnification, are sold on the internet (e.g., amazon.com) for several thousand Japanese Yen. Herein, we evaluated the applicability of a small hand-held microscope with a slide glass stage for field surveys of phytoplankton.

MATERIALS AND METHODS

In March 2014, sample collection using a phytoplankton net (diameter: 20 cm, side length: 80 cm, mesh size: 20 μm) was performed during a shipboard survey around the Selangor river estuary of peninsular Malaysia. A small 40× hand-held microscope (Do-Nature STV-40M, Kenko-Tokina Co. Ltd., Tokyo, Japan) was used for phytoplankton observations on board (Figure 1).

Figure 2 summarizes the steps from plankton sampling to sample observation. On board, a phyto-plankton sample was collected by the vertical hauling of the phytoplankton net (Figure 2A, B). The collected sample was injected from the cock of the net to a 300-mL sample bottle pre-filled with an appropriate amount of surface water (Figure 2C, D). Then, a suitable sample volume was picked up with a disposable dropper, dropped into a glass slide, covered with a cover glass, and observed using the small hand-held microscope (Figure 2E–H). In addition, we attempted to photograph the phytoplankton cells through the eyepiece with a digital camera (Cyber-shot DSC-TX9, Sony, Japan) while on board the ship. However, this was not possible because of the motion of the ship. Therefore, photographs of the sample were taken after the survey while on a jetty. Several photographs were also taken after returning to the laboratory.

RESULTS AND DISCUSSION

Micro-photographs of the phytoplankton observed at the jetty are shown in Figure 3 (40×). Using the hand-held microscope, it was possible to quickly check a wide range of slide glasses and confirm the presence of large phytoplankton. However, the maximum magnification of 40× is a critical limitation.

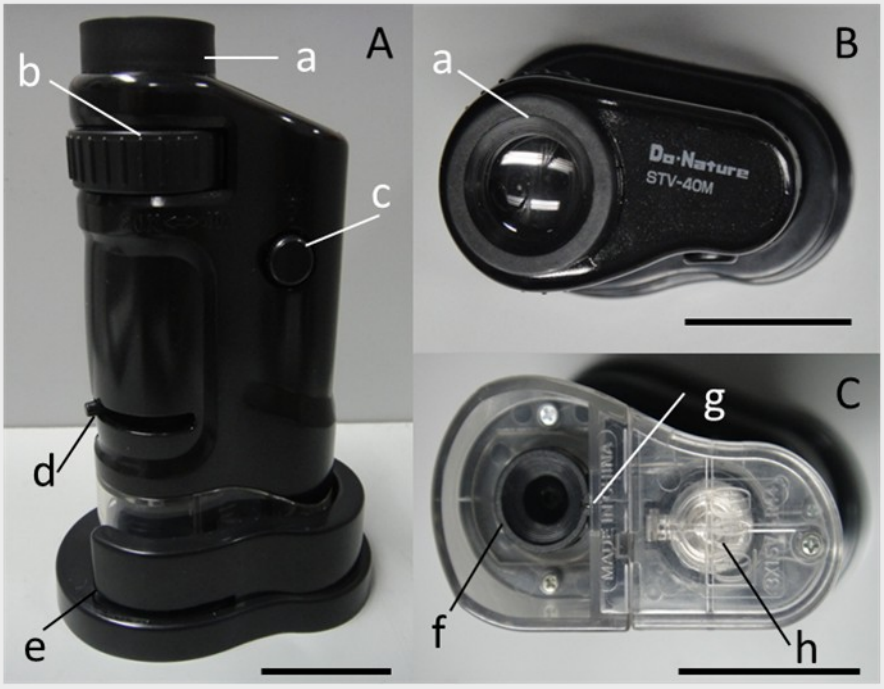

Figure 1: Small hand-held microscope (Do-Nature STV-40M, Kenko-Tokina Co. Ltd., Tokyo, Japan) used for field sampling.
A: side view,
B: top view,
C: lower view.

a: eye lens,
b: zoom,
c: LED lamp switch,
d: diaphragm,
e: sample stage,
f: objective lens,
g: LED lamp,
h: battery. Bar = 25mm.

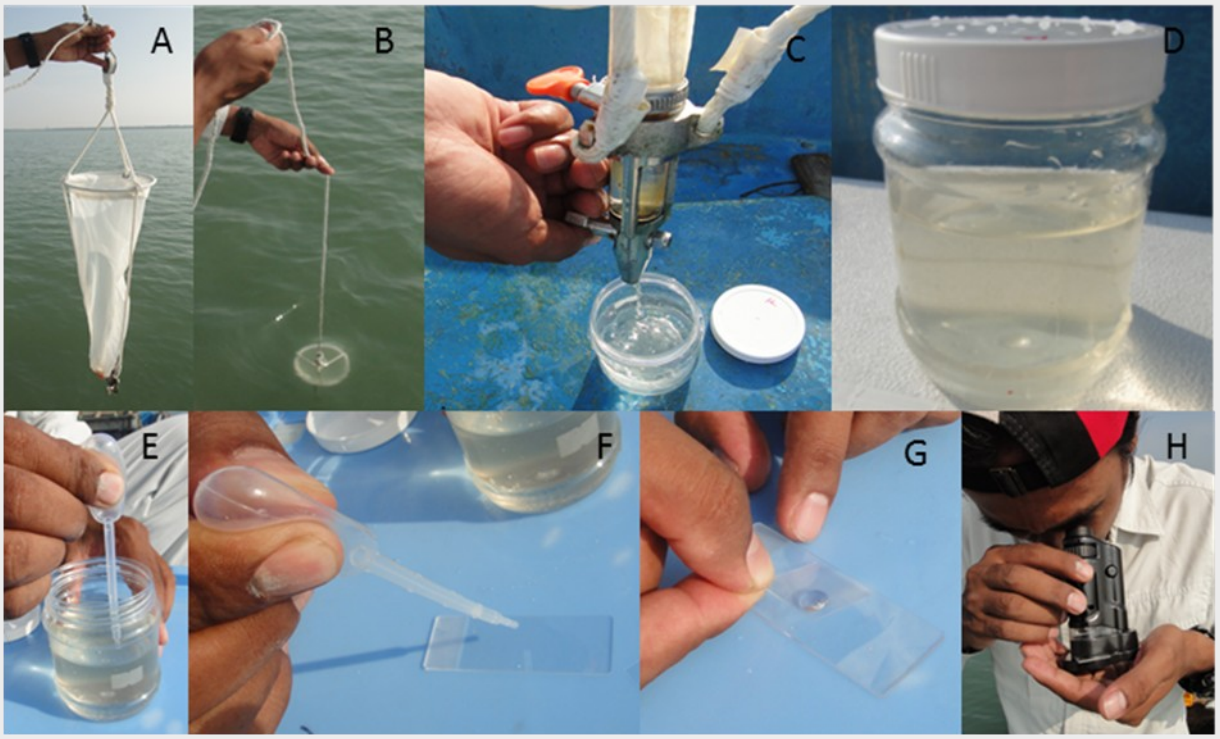

Figure 2: Phytoplankton observation in the field survey using the small hand-held microscope.
A: Phytoplankton net (diameter: 20 cm, side length:80 cm, mesh size: 20μm),
B:Vertical hauling of the plankton net, C: Transfer to the sample bottle from the cock, D:Collected sample, E:Picking the sample up with a dropper, F and G: Making the slide, H: Field observation.

Phytoplankton cells observed included colonial diatom chains such as *Thalassionema* sp. (Figure 3a), *Chaetoceros* sp. (Figure 3b), and *Bacillaria* sp. (Figure 3e) as well as large diatoms such as *Actinoptychus* sp. (Figure 3c) and *Pleurosigma* sp. (Figure 3d). In addition, when the samples were brought back to the laboratory, phytoplankton cells were photographed again using the same microscope and digital camera.

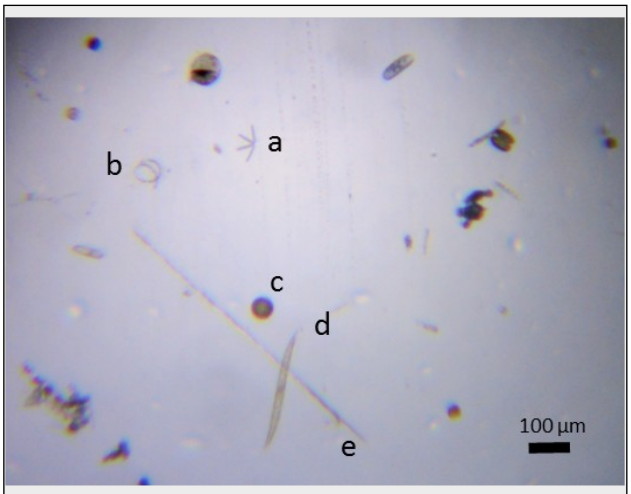

Figure 3: Microphotograph of a collected phytoplankton sample observed with the small hand-held microscope. This photograph was taken while on a jetty, because it was not possible to take photographs on the boat. a:*Thalassionema* sp., b:*Chaetoceros* sp., c:*Actinoptychus* sp., d:*Pleurosigma* sp., e:*Bacillaria* sp. Bar = 100µm

Large diatoms (Figure 4D: *Rhizosolenia* sp.), colonial diatom chains (Figure 4B: *Chaetoceros* sp. and Figure 4G: *Thalassionema* sp.), and small diatoms (Figure 4C: *Odontella* sp., Figure 4F:*Pleurosigma* sp.) were observed in the sample. In addition, 2 species of *Ceratium*, a genus of dinoflagellates, were confirmed (Figure 4A: *Ceratium fusus*, Figure 4E: *Ceratium furca*). The cell size of *Ceratium furca* is smaller than that of *Ceratium fusus*, ranging from 100–200 µm (Fukuyo et al., 1990); moreover, it is larger than the main cell size of toxic or harmful dinoflagellates, which are approximately 50 µm (Fukuyo et al., 1990; Omura et al., 2012). We were unable to observe harmful or toxic

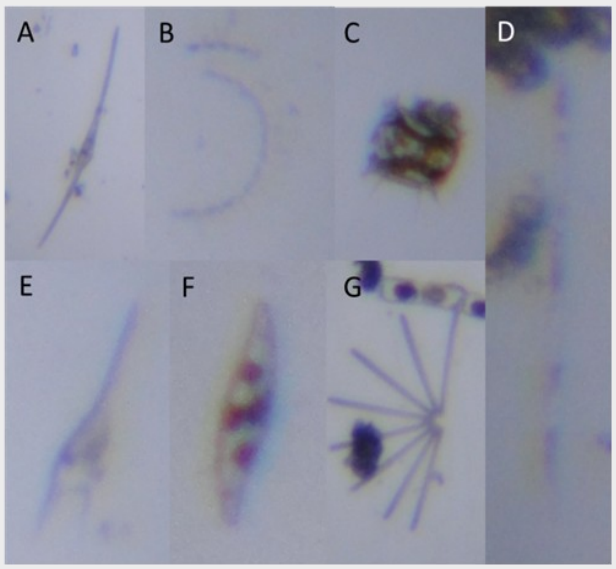

Figure 4: Microphotographs of the collected phytoplankton observed with the small hand-held microscope in the laboratory. A:*Ceratium fusus*, B:*Chaetoceros* sp., C: *Odontella* sp., D:*Rhizosolenia* sp., E: *Ceratium furca*, F:*Pleurosigma* sp., G:*Thalassionema* sp.

dinoflagellate cells in the test sample on this survey. Although we could easily view the samples, it was difficult to observe the details of phytoplankton cells. Nevertheless, small hand-held microscopes are useful tools for phytoplankton observation in field sampling. In addition, for higher magnification, similar hand-held microscopes with an LED lamp but with 60–100× magnification are sold online by other companies, including the HE-18 and LP-33G (Contec Co. Ltd., Japan), a light microscope from Fuji Cosmo Science Co. Ltd., Japan with 60–100×magnification (which appears to not include a slide glass stage), and a small portable microscope including a slide glass stage with 100× magnification (Mizar-Tec, Co. Ltd., Japan); these cost 1,000~3,000 Japanese Yen. In addition, similar microscope with 160–200× magnification with LED lamps can be found for similar prices. Therefore, hand-held microscopes can be selected depending on the application and user preferences. As mentioned above, these small hand-held microscopes are suitable for sample checking in the field. However,

taking photographs with a digital camera in the field was difficult because of a lack of a connecter between the camera and microscope. We are planning to find and evaluate a small hand-held microscope that can be used to take photographs for field surveys.

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