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Comparison of biochemical compositions of phytoplankton during spring and fall seasons in the northern East/Japan Sea

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ABSTRACT

The East/Japan Sea (EJS) where is surrounded by the Korean peninsula, the Japanese islands, and the Russian coast has been experiencing a large change in physicochemical properties. Based on biochemical composition analysis (carbohydrates, proteins, and lipids), the current qualitative status of phytoplankton was identified in the northern EJS from two different sampling seasons (fall and spring in 2012 and 2015, respectively). The average chlorophyll-a (chl-a) concentration integrated from the euphotic depths was significantly higher in 2015 (99.3 ± 69.2 mg m^{-2}) than 2012 (21.5 ± 6.7 mg m^{-2}). Large phytoplankton (> 2 μm) were predominant in 2015 accounting for $64.5 \pm 19.7\%$ whereas small-size phytoplankton (0.7–2 μm) were dominant ($49.1 \pm 17.5\%$) in 2012. The biochemical compositions of phytoplankton were predominated by lipids ($42.6 \pm 7.8\%$) in 2012 whereas carbohydrate composition largely contributed ($53.2 \pm 11.7\%$) to the total biochemical composition in 2015, which is mainly due to different nutrient availabilities and growth stages. Interestingly, the averaged FM concentrations and calorific values for phytoplankton based on the biochemical compositions had similar values between the two years, although the integrated chl-a concentrations were substantially different between 2012 and 2015. In terms of different cell sizes of phytoplankton, we found that small phytoplankton assimilate more FM and calorific energy per unit of chl-a concentration than total phytoplankton. Our results are meaningful for the understanding of future marine ecosystems where small phytoplankton will become dominant at a scenario of ongoing warmer oceans.

1. Introduction

The East/Japan Sea (EJS) is located in the north western Pacific Ocean and semi-enclosed marginal sea surrounded by the Korean peninsula, the Japanese islands, and the Russian coast (Kwak et al., 2013, 2014). Since the various oceanographic features such as mesoscale eddy, subpolar front, deep water formation, and coastal upwelling (Min and Warner, 2005; Riser and Jacobs, 2005; Talley et al., 2006), the EJS has been considered as “Miniature Ocean” (Kim and Kim, 1996; Lee et al., 2009a, 2009b; Kwak et al., 2014).

During the last 50–60 years, the EJS has been experiencing various environmental changes in the physicochemical properties (Kim and Kim, 1996; Kim et al., 2001; Rebstock and Kang, 2003; Kang et al., 2004a, 2004b; Zhang and Gong, 2005; Lee et al., 2009a, 2009b; Gong and Suh, 2012). For example, previous studies reported increasing surface temperature (0.1–0.5 °C), deeper winter mixed layer depth, weaker spring stratification, and increasing pH level: -0.03 ± 0.02 pH units per decade (Kim et al., 2001, 2014; Kang et al., 2003; Chiba et al., 2008). These changes in marine environmental conditions could affect largely marine ecosystem. Especially, the quantitative and qualitative changes in phytoplankton communities could be caused by these environmental changes in the EJS, since phytoplankton as the main group of primary producers are very sensitive to environmental changes. Subsequently, these changes in phytoplankton could induce considerable alternations of upper trophic levels (e.g. recruitment, biomass, and production of fishery resources) (Zhang et al., 2004; Kwak et al., 2013; Lee et al., 2014). Indeed, some studies reported the strong relationship between environmental conditions and biological responses (especially for phytoplankton) in the EJS (Kim et al., 2010; Kwak et al., 2013, 2014; Park et al., 2014). However, their studies mostly focused on the changes in quantitative aspects of phytoplankton (e.g. biomass and primary production) rather than qualitative aspects (e.g. biochemical compositions). Several previous studies on biochemical compositions (carbohydrates, proteins, and lipids) of phytoplankton were conducted

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at various regions (e.g. polar regions, temperate oceans, and coastal regions) (Danovaro et al., 2000; Lee et al., 2009a, 2009b; Lee, 2013; Yun et al., 2015; Kim et al., 2016), but, to date, there is no study about the qualitative changes in phytoplankton community as basic food source in the marine ecosystem of the EJS. The biochemical components of phytoplankton can provide significant information for their physiological and qualitative status. Kim et al. (2016) reported that phytoplankton in the Amundsen Sea, Antarctica, which has been experienced rapid environmental changes (e.g. sea ice coverage and temperature) resulted from mainly the global warming, had a relatively higher proportion of protein content (about 65%) compared to other components. This result indicated that phytoplankton underwent no nitrogen-limiting conditions for their growth period and this high protein contents are believed to provide a high efficiency in carbon transfer to upper trophic levels in the Amundsen Sea (Kim et al., 2016). In the Arctic Ocean case, Yun et al. (2015) observed that low protein and high lipid contents in the biochemical compositions of phytoplankton resulted from nitrogen-deficient growth conditions and showed that an interesting relationship between macromolecular compositions of phytoplankton and biochemical compositions of zooplankton in the northern Chukchi Sea. Thus, it is important to identify the recent physiological status in the phytoplankton community to understand the effects on marine ecosystems, since the phytoplankton could experience considerable changes under the rapidly changing environmental conditions in the EJS.

The primary objective of this study was to identify the current qualitative status of phytoplankton based on their biochemical components in the EJS. The second objective was to understand a seasonal difference of the biochemical composition between two different seasons (fall 2012 and spring 2015). This study will provide important information about the food quality of phytoplankton in the EJS, since this is the first measurement for biochemical compositions of phytoplankton in this region.

2. Materials and methods

2.1. Study area

The samplings were conducted mainly in the northern part of the EJS from two Korea-Russian joint research cruises during the different seasons in 2012 and 2015: one during the fall (13 – 29 October 2012), onboard the R/V *Akademic Oparin*, and the other during the spring (5 April – 2 May 2015) onboard the R/V *Akademic M.A. Lavertiev*. Except a few stations, the major sampling region was located in the northern part of the EJS (Fig. 1). A total of 10 and 11 selected stations were occupied in 2012 and 2015, respectively (Fig. 1 and Table 1).

2.2. Sampling

Temperature and salinity data were obtained by a CTD (SBE 911Plus, Seabird Electronics Inc., Bellevue, USA). Using a CTD-rosette system equipped with 10L Niskin bottles, water samples for chemical and biological measurements were collected from three light depths (100%, 30%, and 1% penetration of surface irradiance, PAR) determined by a secchi disk.

2.3. Chlorophyll-a measurements

Water samples for total chlorophyll-a (chl-a) concentration were filtered through 25 mm GF/F filters (Whatman). In order to estimate relative contributions of different cell-size chl-a (micro-size: > 20 μm; nano-size: 2–20 μm; pico-size: 0.7–2 μm) to total chl-a concentrations, we obtained size-fractionated chl-a samples. One liter of each seawater sample was passed sequentially through 20 and 2 μm Nuclepore membrane filters (47 mm) and then 0.7 μm Whatman GF/F filter (47 mm). The filters were stored in each petri dish which was wrapped with aluminum foil. The filters were kept frozen (– 80 °C) immediately and returned to the Marine Ecological Laboratory at Pusan National University for further analysis. Chl-a for both total and size-fractionated samples was extracted in 90% acetone for 24 h in a freezer (4 °C) and centrifuged, and lastly determined with a Turner Designs model 10-AU fluorometer which had been calibrated by commercially purified chl-a preparations (Parsons et al., 1984).

2.4. Biochemical composition measurements

For the measurement of biochemical composition (carbohydrates, proteins, and lipids) of phytoplankton, water samples (0.5 L) were filtered through 47 mm GF/F filters (Whatman). In order to identify the biochemical compositions of small-size phytoplankton, additional water samples (1 L, only in 2015) were filtered through sequentially 2 μm Nuclepore membrane filters (47 mm) and 0.7 μm Whatman GF/F filters (47 mm). The samples were stored immediately at – 80 °C and the filters were analyzed after returning to the laboratory.

2.4.1. Analysis of carbohydrates

The carbohydrate concentration of phytoplankton was analyzed using the method of Dubois et al. (1956). The filters for carbohydrate analysis were transferred into polypropylene tubes. De-ionized water (1 mL) and 5% phenol (1 mL) were added into the tubes, and then carbohydrates of phytoplankton from the samples were extracted with a sonicator for 20 min. The extracted samples were allowed to keep at room temperature for 40 min. After that, sulfuric acid (H_2SO_4, 5 mL) was put additionally into the solutions. The solutions were homogenized by a vortex mixer and then allowed to stand for 10 min. Upper part of the solutions after centrifuging at 3500 rpm for 10 min were used to determine the carbohydrate concentration of phytoplankton. The absorbance of the carbohydrate sample was measured at 490 nm, using a UV spectrophotometer (Labomed, Germany) with a glucose standard solution (1 mg mL^{-1}, SIGMA).

2.4.2. Analysis of proteins

The protein concentration was measured based on the modified method of Lowry et al. (1951). The samples for proteins were transferred into centrifuge tubes. De-ionized water (1 mL) and alkaline copper solution (5 mL) were added to the tubes, and then the tubes were ultrasonified for 20 min to extract the proteins of phytoplankton. The solutions were mixed with a vortex mixer and stood at room temperature for 10 min. After this process, diluted Folin-Ciocalteu phenol reagent (0.5 mL) was added into each well mixed solution and maintained at room temperature for 1 h 30 min. The solutions were centrifuged at 3000 rpm for 10 min, and then the supernatant of the solution was analyzed to determine the protein concentration with UV spectrophotometer (750 nm). A protein standard solution (2 mg mL^{-1}, SIGMA) was used to calculate the protein concentrations.

2.4.3. Analysis of lipids

The lipid concentration was determined following the modified procedure of Bligh and Dyer (1959), and Marsh and Weinstein (1966). For the lipid analysis, the samples were put into glass tubes with 3 mL mixture of chloroform-methanol (1:2, v/v) and then ultrasonification was done for the lipid extractions from the samples. These glass tubes, which mixed well using a vortex mixer, were stored in the fridge at 4 °C for 1 h. Then, the solvents in glass tubes were centrifuged at 2000 rpm for 10 min and the supernatants were transferred into new tubes. This procedure was repeated again and we merged the later supernatants with the first one for extracting all lipids of phytoplankton from the filters. After adding 4 mL of de-ionized water to the supernatants in new tubes, these solutions were homogenized and centrifuged at 2000 rpm for 10 min. After this process, the solution was divided into two parts; methanol + de-ionized water phase and chloroform phase for lipids. Methanol + de-ionized water phases of each solution were removed