Reply by authors (Mar. 25, 2016) to Anonymous Referee #1 (Received and published: 27 November 2015)

[General Comment]The authors reported interesting observations; clonal culture strains of E. huxleyi changed morphology and size of coccoliths in relation to change in temperature and salinity in laboratory culture experiments. Their observations are very interesting, however, I cannot evaluate accuracy of their experiments at this moment, since they did not describe details of their experiments in the Materials and Methods. Authors did not describe timing of measurements of cell growth rate and of size of coccoliths in their culture experiments, despite it is well known that growth rate of culture strain usually differs greatly between exponentially (logarithmic) and stationary growth phases, and size of coccoliths of E. huxleyi changes in relation to growth phase (growth rate); E. huxleyi make smaller coccoliths in the exponentially growth phase and make larger coccoliths in the stationary phase (Young and Westbroek, 1991). So I am unsure whether the observations on change in coccolith size in this study actually reflect change in temperature and/or salinity, or just reflect change in growth phase.
Another problem; there are too many mistakes in citations. I would recommend authors add detailed information of experiments to materials and methods, reread related papers, and rewrite manuscript with correct references for resubmission.

[Reply] Thanks for the Referee to have pointed out the important fact that we did not clearly describe the timing of the cell growth and of the size of coccoliths. Now, we added the experimental results on cells from both early and late logarithmic growth for MR57N strains as Figure 7 (newly added). Both cell and coccolith sizes at the early logarithmic growth stage were larger than those at the late logarithmic growth stage. In addition, it was difficult to observed coccoliths on the cell at the stationary phase for those species. Therefore, we only compared the cell and coccolith sizes at the early logarithmic conditions.

Another point that the referee gave is also important, so that we did reread the papers and rewrite the manuscript.

Followings are my other comments;
[Comment 1]: Abstract has too much detailed information. The information on the name of the ship used for sampling (line 2), latitude and longitude of sampling localities (line 8), and explanations of classification of morphotype (lines 15-18, the sentence started from According: : :) are unnecessary here.

[Reply 2] We revised the Abstract as suggested by referee.

[Comment 2]: Lines 23-25 of the page 17752 (p.2). Authors wrote "This indicates that subarctic and arctic coccolithophore strains can survive in a wide range of seawater temperatures and at lower salinities due to their marked morphometric adaptation ability" without explaining how 'morphometric adaptation' helps adaptation of E. huxleyi to various temperature/salinity conditions. Please explain it in the Discussion.

[Reply 2] Sorry for such misleading sentence. Now, we changed to:
"This indicates that subarctic and arctic coccolithophore strains can survive in a wide range of temperatures and low salinities with change in their morphology." (p. 2)

[Comment 3]: Lines 14-16 of the page 17753 (p. 3); Please describe the definition of 'warm water' and 'cold water' in the studied area (Arctic Ocean and Bering Sea).

[Reply 3] We added the information requested. So, "warm water" is ~5°C, while "cold water" is <0°C. (p. 3)

[Comment 4]: Line 18 of the page 17753 (p. 3); Prymnesiophyceae not Prymneophyceae. More correctly, E. huxleyi belongs to the Family Noelaerhabdaceae, Order Isochrysidales, Class Prymnesiophyceae not to Prymneophyceae family.

[Reply 3] Thanks. We corrected to "Prymnesiophyceae". (p. 3)

[Comment 5]: Lines 1-9 of the page 17754 (p. 4); Citations in these sentence are wrong. Authors wrote "Hagino et al. (2011) classified coccolith morphotype into four groups: (1) Type A and Type R with: : :..". Correctly, Hagino et al. (2011) classified E. huxleyi into seven groups! Therefore, the all explanations concerning morphotypes of Hagino et al. (2011) in these sentences are inaccurate.

[Reply 5] Thanks for valuable comments and sorry for such a mistake. Now, we revised the text to "Hagino et al. (2011) classified coccolith morphotype into seven types, and further grouped into the four cross-sectioned types". (p. 4)

[Comment 6]: Line 4 of the page 17754; 'corona' should be written in italic.

[Reply 6] Thanks. We revised it on the line 4 and 8, as suggested.

[Comment 7]: Line 14 of the page 17754; 'McIntyre and Bé' not 'McIntyre and Be'

[Reply 7] We collected it in all parts of the text and reference.

[Comment 8]: Lines 14-16 of the page 17754; Citations in these sentences are completely wrong. Authors wrote "According to McIntyre and Be (1967), Type A and Type C likely correspond to warm- and cold-water types, respectively, although Hagino et al. (2011) reported that Type C has not always been reported in cold-water environments". Correctly, McIntyre and Bé (1967) just described warm and cold types of E. huxleyi. Young and Westbroek (1991) renamed warm and cold types of McIntyre and Bé (1967) as Types A and C, respectively. They renamed the morphotypes of E. huxleyi since Winter (1987) mentioned cold type (= Type C in Young and Westbroek 1991) was not always related to low temperature. Hagino et al. (2000) and Hagino et al. (2006) reported type C from tropical area, but Hagino et al. (2011) did not. Hagino et al. (2011) just introduced observation by Winter (1987) and interpretation by Young and Westbroek (1991).

[Reply 8] Thanks for valuable comments and sorry for such a misleading description. Now, we revised the sentences to: "Concerning the oceanographic distribution of Type A and Type C, defined by Young and Westbroek (1991), approximately correspond to warm- and cold-water types, described by McIntyre and Bé (1967), respectively, although Type C has not always been reported in cold-water environments (Young and Westbroek, 1991; Hagino et al., 2011)."

[Comment 9]: 2. Materials and methods; Please provide more detailed information on materials and methods of experiments.

[Reply 9] As requested, we added the more detailed information of the timing of the growth and the measurements of the sizes in "Materials and methods" and also "Results".

[Comment 10]: Line 12 of the page 17755; How did you collect 'samples' that yielded your culture strains? Please show in situ seawater temperature and salinity of the water samples that yielded culture strains used in this study.

[Reply 10] We added data on *in situ* seawater temperature and salinity of the water samples like the below database:

[Comment 11]: Line 17 of the page 17755; How did you establish clonal culture strain from your 'samples'?

[Reply 11] We added detailed description in the text (see Materials and methods) and also added a reference, Satoh et al., 2013.

[Comment 12]: Lines 20-21 of the page 17755; Authors wrote "The growth rate at each temperature was calculated as the average value of triplicate experiments, and the error bars indicated the minimum and maximum values." I think the growth rate of E. huxleyi is usually changes during culture experiments. Please describe the detailed method used for monitoring of growth rate in this study, and provide information of growth phase of each culture strain at the timing of sampling for the studies of growth rate.

[Reply 12] As shown in Fig. 1, growth profiles in triplicate experiments were quite similar.

To determine the timing of sampling for SEM observation, we firstly compared cellular SEM images between the early and the late logarithmic growth phase using MR57N stains (see Supplemental data or Fig. 7 (new)). The results indicated that both cell and coccolith sizes were larger in cells harvested at the early than the late logarithmic growth phase Thus, we used cells harvested at the early logarithmic growth phase even for other strains.

[Comment 13]: Line 26 of the page 17755; How did you know the strain MS1 is type A?

[Reply 13] Hagino et al. (2011) described that MS1 is Type A. In addition, we also observed the curved central in MS1 (same as RCC1226) under SEM. Now, we revised the text by adding a reference of Hagino et al. (2011) in the text.

[Comment 14]: Line 26 of the page 17755; The strain code of MS1 in the Roscoff culture collection is RCC 1226 not D2801-5.

[Reply 14] D2801-5 is another name of MS-1, so that we changed to strain number "RCC1226"

in the text.

[Comment 15]: Line 1 of the page 17756; How did you know the NIES 1311 is type O?

[Reply 15] Hagino et al. (2011) reported that NIES 1311 is type O. According to our SEM observation, the central area of NIES1311 is opened and agreed with Hagino et al. (2011). Now, we revised the text by adding the reference, Hagino et al. (2011).

[Comment 16]: Line 24 of the page 17756; How did you prepare sample for measurement of cell density in a polarized microscope?

[Reply 16] We added more detailed procedure in the text;
"The numbers of cells in 10 μL cell suspension, including both calcified and non-calcified (naked) cells, were determined using cell counting glass plate under a polarized microscope equipped with camera system and a calibration curve guaranteed by cell counting using the Thoma's haemocytometer. "

[Comment 17]: Lines 26-29 of the page 17756; Please describe pore size, diameter, and product name of the polycarbonate filter.

[Reply 17] We used a Isopore membrane filter with 0.8 μm pore size, a Millipore product, ATTP04700. These information was added to the text.

[Comment 18]: Young and Westbroek (1991) reported size of coccoliths of E. huxleyi changes in culture experiments in relation to growth phase. Please provide information of growth phase of each culture strain at the timing of sampling for morphometric studies under SEM.

[Reply 18] The timing of sampling for morphometric studies were performed at the early logarithmic growth stage, mostly the 3rd day of the growth experiments, because of the below two reasons. Firstly, the reason is that the size of the coccolith and cell were larger at the early logarithmic stage for MR57N strains. Secondly, it is difficult to observe the coccoliths on the cell at the later steady growth stage using SEM.

[Comment 19]: Lines 2-10 of the page 17759; Young and Westbroek (1991) and Young et al. (2003) mentioned that central area of Type A consists of 'curved elements', while that

of Type B (and B/C) consists of 'lath-like elements'. Authors classified their culture strains into type B/C without description of morphology of central area elements. So I am unsure if their strains are actually type B/C or not. Please describe morphology of central area elements of the culture strains used in this study.

[Reply 19] MR strains show "lath-like" central area, so that it is consistent with Young and Westbroek (1991) and Young et al. (2003).

[Comment 20]: Lines 10-11 of the page 17762; What is the 'scaling low'?

[Reply 20] "Scaling low" means that the morphometrical parameters are directly proportional.

[Comment 21]: Lines 22-23 of the page 17762 "On the other hand, Types A and B were found around the Southern Subtropical Front in a warm-water areas." Please provide information on papers that reported Type B from warm-water area.

[Reply 21] we added the reference, Patil et al. (2014).

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Reply to Reviewer #2:

Thank you very much for your positive evaluation.

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