Korean Journal of Remote Sensing, Vol.18, No.1, 2002, pp.35~42

Validation of chlorophyll algorithm in Ulleung Basin, East/Japan Sea

Sin-Jae Yoo*, Hyun-Cheol Kim*, Jeong-ah Lee*, and Mi-Ok Park**

Korea Ocean Research and Development Institute, South Korea*

Pukyung National University, South Korea**

Abstract : The results of our observation in May 2000 indicated that the SeaWiFS algorithm (O'Reilley *et al.*, 1998), which was adopted for OSMI data processing, overestimated the actual chlorophyll values. This was rather unexpected in that there were good reasons to expect that the bio-optical properties of East/Japan Sea belonged to Case 1 water and in such case, the OC2 algorithm would give unbiased estimates of actual chlorophyll a values. In November 2000, a cruise conducted bio-optical surveys in the same area. This time we added HPLC (High Performance Liquid Chromatography) method for measuring chlorophyll *a* concentration to the standard fluorometric method, which we have been using during the past. Fluorometric method with acidification is known to result in under/overestimation of chlorophyll values in many parts of the world oceans, while it is easier and cheaper than HPLC method. To our surprise, the comparison of HPLC chlorophyll and fluorometric chlorophyll values show that fluorometric values gave an underestimation up to 50%. This error was due to the presence of accessory pigments such as chlorophyll *b*. Considering this error, our previous result of May 2000 (Yoo *et al.*, 2000) might have to be reinterpreted. Calculation of reflectance at 490 and 555nm, however, indicated that this is not still enough to explain the discrepancies.

Key Words : OC2 Algorithm, OSMI, HPLC, Chlorophyll *a*, Ulleung Basin, Pigments.

1. Introduction

There are good reasons to presume that the optical properties of the East Sea/Japan Sea would belong to case 1 water: There is little direct drainage of significant river discharge; The extent of continental shelf is insignificant and the tidal range is small (~0.2m). As a consequence, little input of terrigenous materials into the basin is expected. The results of our observation in Ulleung Basin in May 2000 indicated that at least for the Ulleung Basin, this might not be the case (Yoo *et al.*, 2000). OC2 algorithm (O'Reilley *et al.*, 1998), when applied to in situ reflectance, overestimated the *in situ* chlorophyll values. We suggested that entrainment of coastal waters into Ulleung Basin by East Korean Warm Current might have altered the optical properties of the Ulleung Basin water (Yoo *et al.*, 2000).

However, such deviation could have also resulted if

Received 31 December 2001; Accepted 30 January 2002.

chlorophyll *a* concentrations were underestimated. It has been known that the most widely used method of chlorophyll *a* quantification, i.e., fluorometric method with acidification, could either overestimate or underestimate chlorophyll *a* (Loftus and Carpenter, 1971; Trees *et al.*, 1985; Smith, 1987; Bianchi *et al.*, 1995). Therefore, errors in chlorophyll *a* determination might still further increase the deviation. In November 2000, a cruise was conducted in the same region, and this time, HPLC (High Performance Liquid Chromatography) measurements were made for pigment analysis in addition to the standard fluorometric measurements of chlorophyll *a*. Here we report the results of the survey.

2. Materials and methods

Samples were obtained from 22 stations during Nov 3 ~ Nov 10, 2000 (Fig. 1). Samples were collected in Niskin bottles, and filtered through GF/F glass fiber filters. Sample volume was 1 liter for fluorometric analysis and 1.5 ~ 3.0 liter for HPLC pigments analysis depending on chlorophyll level. The filters were immediately stored in liquefied nitrogen until analysis. For fluorometric analysis, a Turner Designs 10-005R fluorometer equipped with standard lamp and excitation/emission filters for chlorophyll *a* analysis was used. Acidification method (Parsons *et al.*, 1984) was applied to separate chlorophyll *a* and phaeopigment *a*.

For HPLC analysis, an Orom Vintage 2000

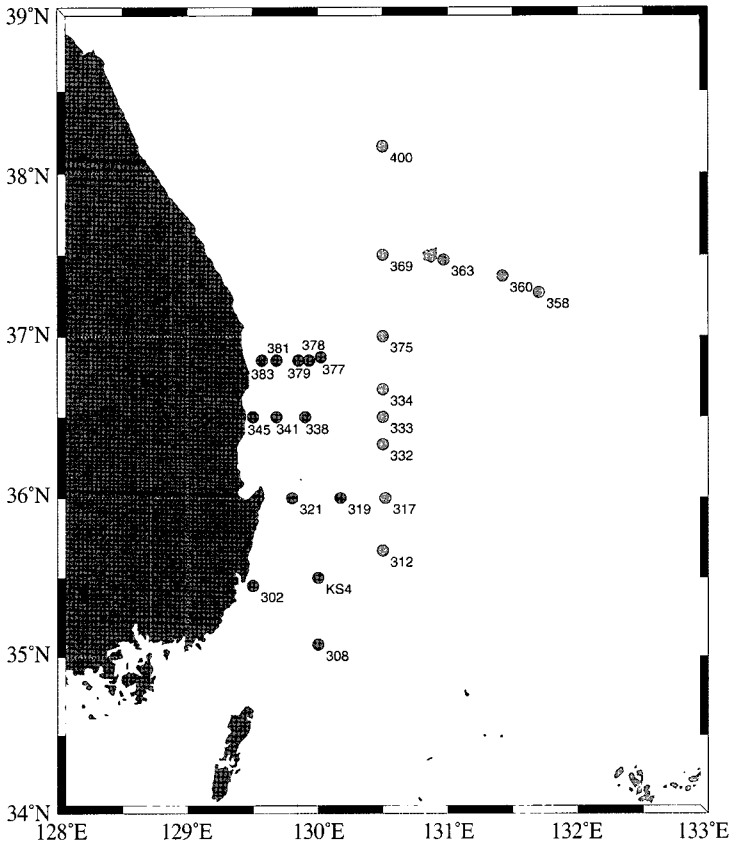

Fig. 1. Stations where pigment analysis was made in Nov 2000 cruise.

(Oromtech, Korea) equipped with Rexchrom S5-100-ODS column (OctaDecy-Silane bonded phage, Regis, 250 × 4.6mm, 5μm particle size, USA) was used. Absorbance detector (at 436nm) used was UV-200 (Oromtech, Korea). Spectrofluorescence detector used was RF-551 (excitation = 432nm; emission = 650nm; Shimadzu, Japan). Three solvents were used in programmed gradient elution mode: solvent A (MeOH 80%, ammonium acetate 0.5M 20%, BHT 0.01%), solvent B (acetonitrile 87.5%, H_2O 12.5%, BHT 0.01%), and solvent C (ethyl acetate 100%). Pigments quantified were chlorophyll *a* (mono + divinyl), phaeophytin *a*, chlorophyll *b* (mono + divinyl), chlorophyll *c* (*c*1 + *c*2), alloxanthin, 19'-butanoyloxy-fucoxanthin, beta-carotene, diadino-xanthin, fucoxanthin, peridinin, prasinoxanthin, vio-laxanthin, lutein, and zeaxanthin.

3. Results

HPLC chlorophyll *a* concentrations ranged from 0.36 (station 319) to 2.66 mg m^{-3} (station 381). The proportion of total accessory pigments was rather constant ranging 39.1 ~ 46.3% of the total pigments quantified. One typical example of station 334 is shown in Fig. 2(a). Carotenoids accounted for 15.7 ~ 25.2% in all the stations. The composition of carotenoids at station 334 is shown in Fig. 2(b). Of all the measured pigments, chlorophyll *b* accounted for 5.96 ~ 12.75%. The ratio of chlorophyll *b* to chlorophyll *a* was about 2.4 ~ 25.7%, while that of chlorophyll *c* to chlorophyll *a* ranged 13.3 ~ 26.4%, while chlorophyll *b* and *c* (*c*1 + *c*2) showed a high correlation with chlorophyll *a*, 19'-butanoyloxy-fucoxanthin showed a negative correlation with chlorophyll *a*. Zeaxanthin showed no correlation with

chlorophyll *a*. Comparison of fluorometric chloro phyll *a* and HPLC chlorophyll *a* from 29 samples (22 stations) showed that fluorometric method under-estimated HPLC chlorophyll *a* by 14 ~ 59% (Fig. 3). Slope of the linear regression was 0.50 and y intercept was 0.0293 (R^2 = 0.933). Some authors (e.g., Bianchi *et al.*, 1995) attributed the under-estimation to the chlorophyll *b*. They also found that in some stations, overestimation was resulted by high chlorophyll *c*. In this study, there was a linear relationship between the

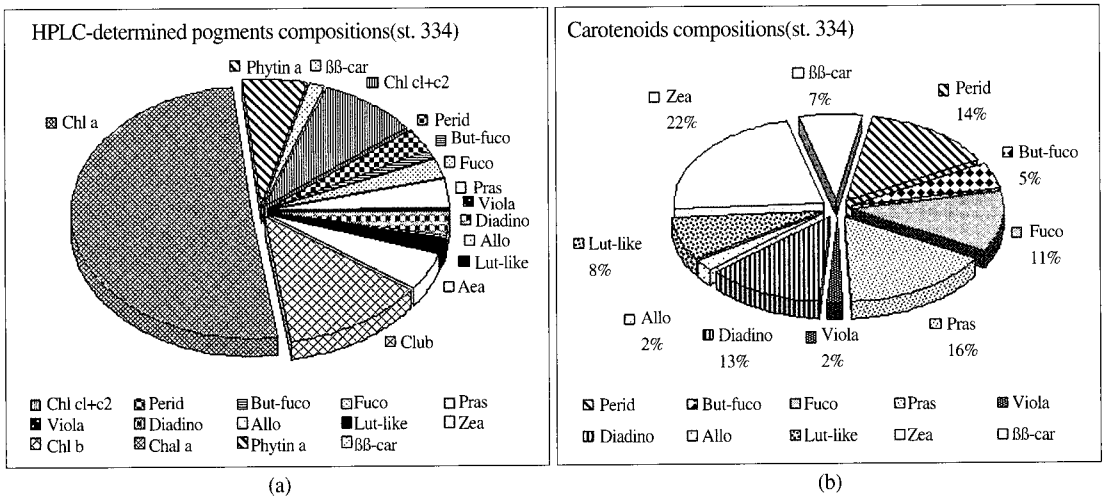

Fig. 2. (a) A typical example of pigment composition (station 334). Chlorophyll *a* accounted for more than half of the pigments in all the stations. (b) Composition of carotenoids in station 334: While each pigment concentration was variable between stations, the total carotenoids were rather constant.

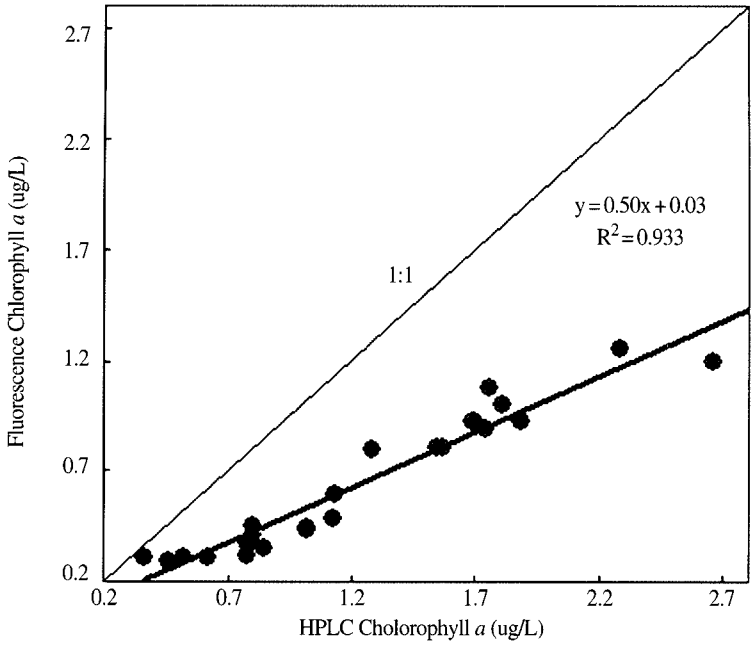

Fig. 3. Comparison of fluorometric chlorophyll *a* and HPLC chlorophyll *a*.

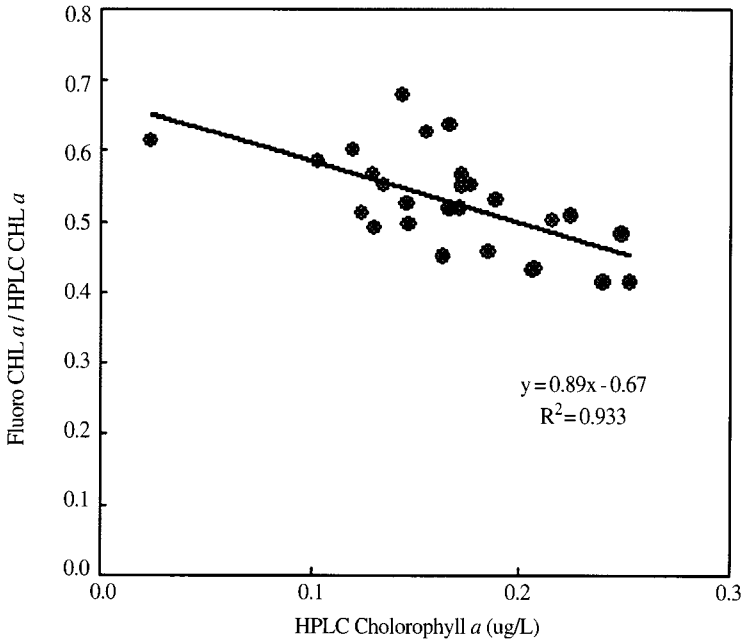

Fig. 4. Relationship of HPLC chlorophyll *b*/chlorophyll *a* ratio with fluorometric chlorophyll *a*/HPLC chlorophyll *a* ratio.

error of estimation (the ratio of fluorometric chlorophyll *a* to HPLC chlorophyll *a*) and the ratio of chlorophyll *b* to chlorophyll *a* so that as the chlorophyll *b* increased relative to chlorophyll *a*, there was a tendency of increasing underestimation (Fig. 4). This variability of the ratio represented the change in the composition of

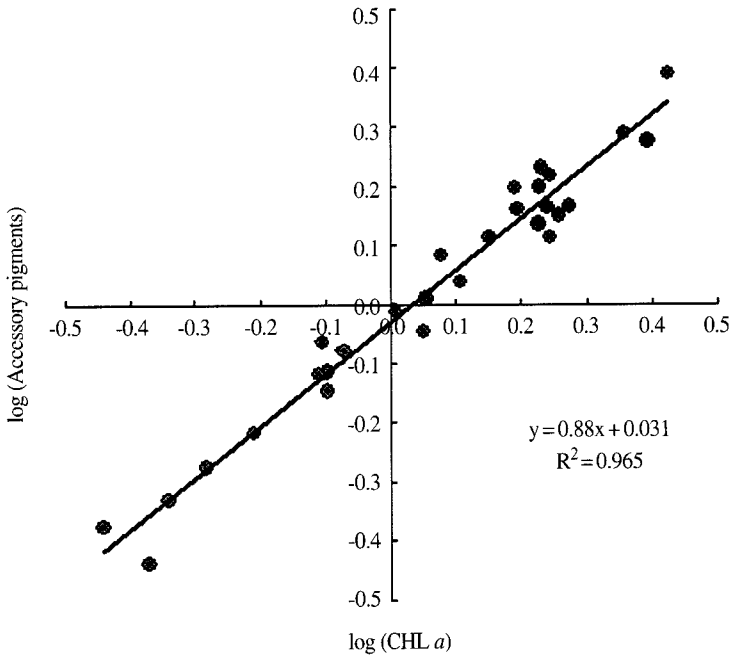

Fig. 5. Relationship between logarithm of chlorophyll *a* concentrations and logarithm of total accessory pigment concentrations.

taxonomic groups in the phytoplankton, particularly, the proportion of pico-plankton.

While the ratio of chlorophyll *b* to chlorophyll *a* was variable, there was a consistent relationship between the logarithm of chlorophyll *a* con-centrations and the logarithm of total accessory pigment concentrations (Fig. 5). Trees *et al.* (2000) found a remarkable relationship between log [chlorophyll *a*] and log [total accessory pigments] from a global dataset of 5,617 samples that represented widely different environments (slope = 0.934, y intercept = 0.028, R^2 = 0.946). Although the kinds of accessory pigments analyzed here were slightly different from their analysis, our results were comparable.

4. Discussion

In the previous study in May 2000 (Yoo *et al.*, 2000), we found the currently adopted chlorophyll algorithm resulted in substantial overestimation compared to *in situ* chlorophyll concentrations. Such overestimation results from the deviation from the expected relationship between the ratio of R(490)/R(555) and chlorophyll *a* concentrations (circles in Fig. 5). All of our 23 data had lower R(490)/R(555) values than expected values by OC2 algorithm, given chlorophyll *a* values. We postulated that additional absorption of CDOM (Colored Dissolved Organic Matters) could result in lower R(490)/R(555) values. Fig. 7 shows the composition of absorption in the stations of May 2000 survey. It shows that the CDOM absorption is greater at 490 nm in magnitude and proportion than at 555 nm and thereby its overall effects were to decrease R(490)/R(555).

However, this seems not sufficient to explain the deviation. We re-calculated R(490)/R(555) by subtracting absorption due to CDOM (asterisks in Fig. 6). In this calculation we ignored suspended sediments as we did not have data of absorption or backscattering of suspended sediments. The results indicate that there is still unexplained deviation (Fig. 6).

The comparison of standard fluorometric chlorophyll

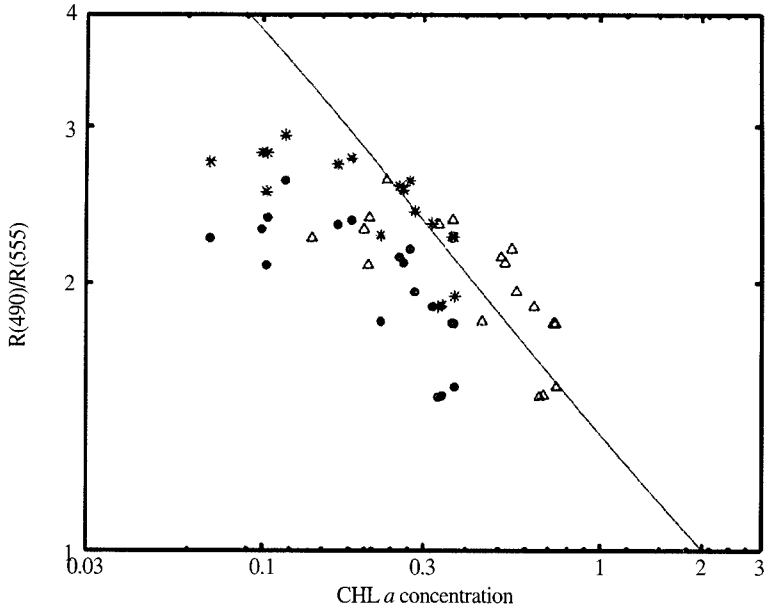

Fig. 6. Relationship between R(490)/R(555) vs. chlorophyll *a* concentrations from May 2000 survey. The line is the expected relationship by OC2 chlorophyll algorithm. Circles are original data. Triangles are when chlorophyll *a* values are multiplied by 2.0. Asterisks are when reflectance ratios are recalculated after CDOM absorption was subtracted.

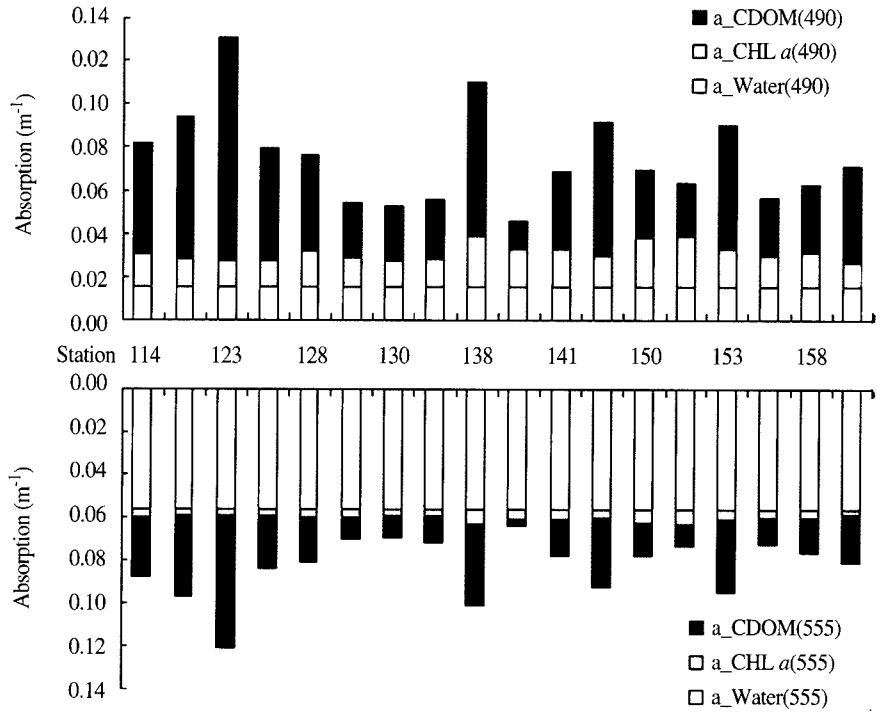

Fig. 7. Composition of absorption in May 2000 stations. CDOM absorption is greater at 490nm in magnitude and proportion than at 555 nm.

a measurements and HPLC chlorophyll *a* in this study indicates that errors in chlorophyll *a* might also have contributed to the underestimation. Unfortunately, no optic measurements were available in Nov 2000 cruise due to unfavorable weather so that direct comparison of R(490)/R(555) and chlorophyll *a* was not possible. In Fig. 6, we adjusted chlorophyll *a* of May 2000 data by multiplying 2.0 assuming the errors in May 2000 were similar to Nov 2000. This adjustment pushed the points to the right side (triangles in Fig. 6) making the fit better.

However, this adjustment alone cannot explain the deviation. The magnitude of errors was not likely this much in May 2000. Since it was the late phase of spring bloom and the proportion of non-diatom cells that cause the underestimation might not have been significant. On the other hand, the proportion of picoplankton in terms of chlorophyll *a* ranged 41.9 ~ 63.9% in Nov 2000 (J. H. Noh, personal communication).

The magnitude of underestimation error of this study was rather high with an average of 45.8%. Jacobsen (1978) reported a wide range of error from −59 to +78% in fluorometric determination of chlorophyll *a*. Trees *et al.* (1985) in a more extensive study based on three cruises reported 28 ~ 47% of underestimation error. Bianchi *et al.* (1995) found an average error of 30% for underestimation of chlorophyll *a* using standard fluorometric method from 65 samples in the continental margin of northwestern Gulf of Mexico. Therefore, there seems a wide range of variability in either over- or underestimation by fluorometric method. The variability will be dependent upon the composition of algal pigments. HPLC analysis will be useful in providing information on phytoplankton pigments and dominant taxonomic groups (Liaaen-Jensen, 1985).

5. Conclusions

Application of ocean color satellite data requires the data accuracy as a prerequisite. How good the retrieval of level 1 and level 2 products from ocean color sensors is, therefore, a fundamental procedure. This study was conducted to evaluate the accuracy of standard ocean color algorithm. In particular, we tried to evaluate the accuracy of global in-water algorithms when applied to coastal seas. Our results showed that the errors of chlorophyll *a* estimation by OC2 algorithm in May 2000 might have resulted from errors in chlorophyll *a* quantification as well as from the fact that the optical properties of Ulleung Basin water deviated from those of Case 1 water.

The error in the standard fluorometric method should be checked for different seasons as well, since nature (over- or under-estimation) and magnitude of such error will be dependent upon the dominant algal groups with particular accessory pigments. For example, green algae (Chlorophyceae and Prasino-phyceae) contain chlorophyll *b*, while diatoms and coccolithophorids contain chlorophyll *c*.

In addition, our unpublished data indicate that the concentrations of suspended sediments in this study were not negligible (mean = 2.48 mg l^{-1}). Therefore, the optical properties of Ulleung Basin should be studied further including the contribution of suspended sediments in absorption and backscatter.

Acknowledgements

This study was conducted as a part of MOST project "Validation of OSMI and its marine application".

References

Bianchi, T. S., C. Lambert, and D. C. Biggs, 1995. Distribution of chlorophyll *a* and phaeopigments in the northwestern Gulf of Mexico: a

comparison between fluorometric and high-performance liquid chromatography measurements, *Bull. Mar. Science*, 56: 25-32.

Jacobsen, T. R., 1978. A quantitative method for the separation of chlorophyll *a* and *b* from phytoplankton pigments by high-pressure liquid chromatography, *Mar. Sci. Commun.*, 4: 33-47.

Liaaen-Jensen, S., 1985. Carotenoids of lower plants: Recent progress, *Pure Appl. Chem.*, 57: 649-658.

Loftus, M. E., and J. H. Carpenter, 1971. A fluorometric method for determining chlorophyll a, b, c, *J. Mar. Res.*, 29: 319-338.

O'Reilly, J.O., S. Maritorena, B.G. Mitchell, D.A. Siegel, K.L. Carder, S.A. Garver, M. Karhu, and C. McClain, 1998. Ocean Color chlorophyll algorithms for SeaWiFS, *J. Geophys. Res.*, 103 (24): 937-24953.

Parsons, T. R., Y. Maita, and C. M. Lalli, 1984. A manual of chemical and biological methods for seawater analysis, *Pergamon press, Oxford.*

Smith, R. C., R. R. Bidigare, B. B. Prézelin, K. S. Baker, and J. M. Brooks, 1987. Optical characterization of primary productivity across a coastal front, *Mar. Biol.*, 96: 575-591.

Trees, C. C., D. K. Clark, R. R. Bidigare, M. E. Ondrusek, and J. L. Mueller, 2000. Accessory pigments versus chlorophyll a concentrations within the euphotic zone: a ubiquitous relationship, *Limnol. Oceanogr.*, 45:1130-1143.

Trees, C. C., M. C. Kennicutt II, and J. M. Brooks, 1985. Errors associated with the standard fluorimetric determination of chlorophylls and phaeopigments, *Mar. Chem.*, 17: 1-12.

Yoo, S., J. Park, and H. Kim, 2000. Validation of ocean color algorithms in the Ulleung Basin, East/Japan Sea, *J. Korean Soc. Remote Sensing*, 16(4): 315-3