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Memorandum Report **NURC-MR-2011-001/Ed.2**

Spectrofluorometric and HPLC chlorophyll–α measurement comparison

Marina Ampolo Rella

May 2011

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One of three research and technology organisations in NATO, NURC conducts maritime research in support of NATO's operational and transformation requirements. Reporting to the Supreme Allied Commander, Transformation and under the guidance of the NATO Conference of National Armaments Directors and the NATO Military Committee, our focus is on the undersea domain and on solutions to maritime security problems.

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Spectrofluorometric and HPLC chlorophyll-α measurement comparison

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This document, which describes work performed under Project EKOE (Environmental Knowledge and Operational Effectiveness) of the NURC Scientific Programme of Work, has been approved by the Director.

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Spectrofluorometric and HPLC Chlorophyll-α measurement comparison.

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Abstract: To address uncertainties inherent in fluorescence in vitro analysis and the measurement of chlorophyll-α, this study was conducted to develop a set of cross-referenced samples analyzed using both spectrofluorometry and high performance liquid chromatography (HPLC). A linear regression was developed between the two methodologies, and coefficients are now being applied to spectrofluorometric measurements to improve chlorophyll-α data results at the NATO Undersea Research Centre (NURC) Oceanographic Branch Laboratory.

Keywords: Spectrofluorometer, HPLC, chlorophyll-α

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Preface

This is the second edition of NURC-MR-2011-001, originally published in January 2011. For convenience of holders of the first edition, the following is a list indicating the pages (in the first edition) which have been modified:

p. 5: Plot axes reversed and axes definition changed accordingly.
p. 7: Plot scale is now linear.

1 Introduction

The uncertainties related to the fluorescence *in vitro* analysis and the given possibility of having a set of cross referenced samples analyzed using both methodologies, fluorometric and High Performance Liquid Chromatography, give rise to this Laboratory report in which a confident linear regression between the two has resulted in internal coefficients being applied to every spectrofluorometric measurement performed in the NURC Oceanographic Branch laboratory.

Over 250 samples were gathered during NURC cruises in 2008 and 2009. These samples were analyzed to define the reliability of the in-house spectrofluorometric method and to develop corrections.

2
Material and Method

HPLC and spectrofluorometric samples were collected from the same station, water depth, and Niskin bottle. For continuity, samples were filtered and prepared using the same equipment and by the same operator. Samples were collected on GF/F filters, 25 mm for fluorescence and 47 mm for HPLC. The first samples were analyzed on board after the required extraction time; the second samples were stored in liquid nitrogen and then shipped to Analytical Services HPL-UMCES (Cambridge, MD, US) for analysis.

The spectrofluorometric method used was Holm-Hansen et al. (1965) with further modifications.

$Ca = (Rmax / (Rmax -1))*(Rb-Ra)* \mathbf{f} *Vex/ Vfilt$ [1]

$Cp = (Rmax / Rmax -1))*((Rmax *Ra) - Rb)* \mathbf{f} *Vex/Vfilt$ [1]

A Varian Cary Eclipse spectrofluorometer was used for the readings. The instrument was routinely serviced by the manufacturer (cleaning, stability, alignment checks and adjustments) and calibrated in-house using a pure chlorophyll-α standard dissolved in acetone (DHI Lab Products, Denmark). Values derived from the standard dilutions are double checked using a Perking Elmer spectrophotometer with a 1-cm cuvette.

Final concentration is then retrieved and compared with the certified value.

$Cstd = 106 [A (\lambda max) – A (750)] / bE1cm$ [1]

The spectrofluorometer is calibrated using a concentration vs. instrument response curve. In the last 5 years, the instrument has proven to be stable (Table 1).

Table 1: Response factors and acidification ratios computed from calibrations

Year	Rmax	*f*
2005	8.296	0.708
2005	7.889	0.613
2006	6.937	0.632
2007	7.101	0.693
2008	7.376	0.754
2009	6.849	0.786

Samples were taken during five cruises in different areas and seasons (Table 2). In this way population variety, growth phase, and other factors are taken into account.

Table 2: Cruise information

Cruise	Area	Date
TSS08	Aegean, Marmara and Black Seas	Sep. 2008
LSCV08	Ligurian Sea	Oct. 2008
TSS09	Marmara and Black Seas	Jan. 2009
BP09	Ligurian Sea	Mar. 2009
MED09	Alboran Sea	Aug. 2009

3 Results

Results show strongly correlating trends between the two methodologies; however, the spectrofluorometric method underestimates the real value of chlorophyll-α with a nearly constant error. Data were plotted and a standard linear regression fit was applied. After this first step, the data error related to this first interpolation is computed. For the final formula, only data included in the median of the error distribution were used. The equation below describes the linear regression calculated on the final data set. It corrects the chlorophyll-α values retrieved from the spectrofluorometric analysis.

$$y = 1.8209x - 0.0056$$

$$R^2 = 0.98$$

where:

x = spectrofluorometric chlorophyll-α value in µg/l

y = HPLC chlorophyll-α value in µg/l

R^2= Coefficient of determination

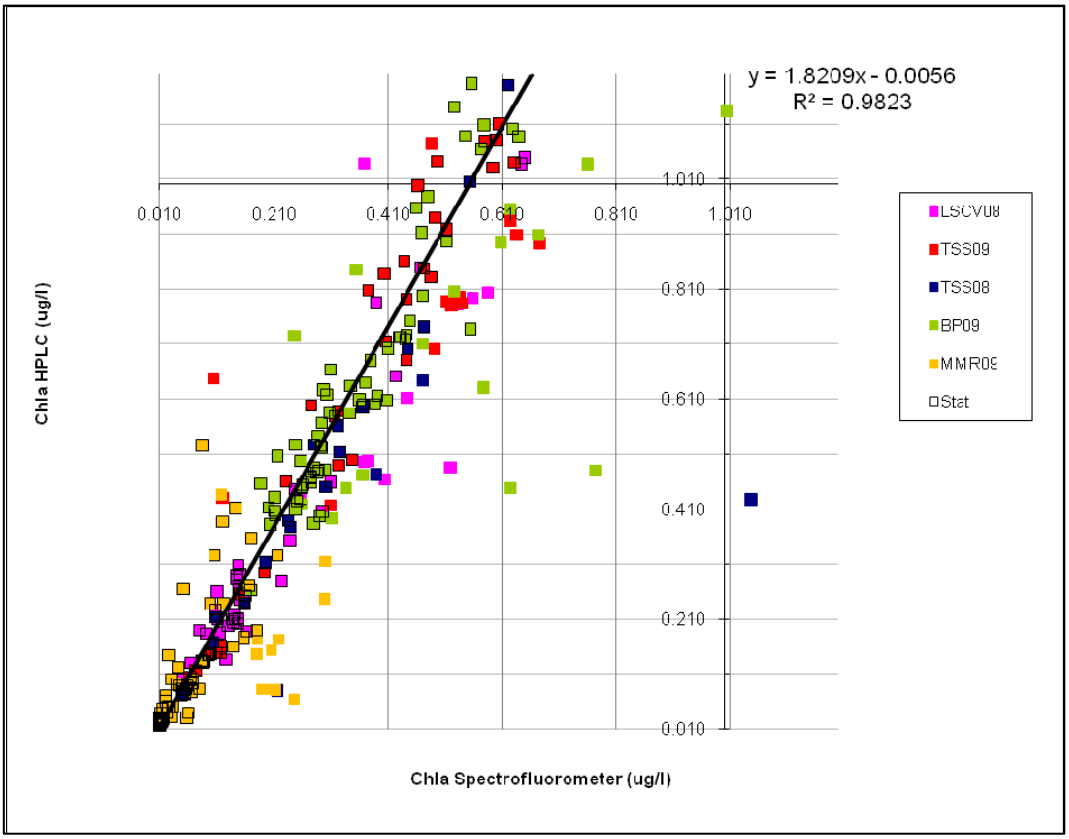
These coefficients must be applied to the in-house spectrofluorometric chlorophyll-α results to achieve higher accuracy of data.

Acknowledgements

This work merges data acquired under different NURC scientific projects. The support of S. Besiktepe, J. Haun, and C. Trees (NURC Scientists-in-Charge) and Analytical Services HPL-UMCES (Cambridge, MD, US)is acknowledged.

Annex

The following figure shows chlorophyll-α data (in µg/l) obtained with the two methods.



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Title

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Keywords

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