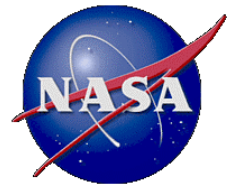


HPLC Analysis of Phytoplankton Pigments Protocol: Update

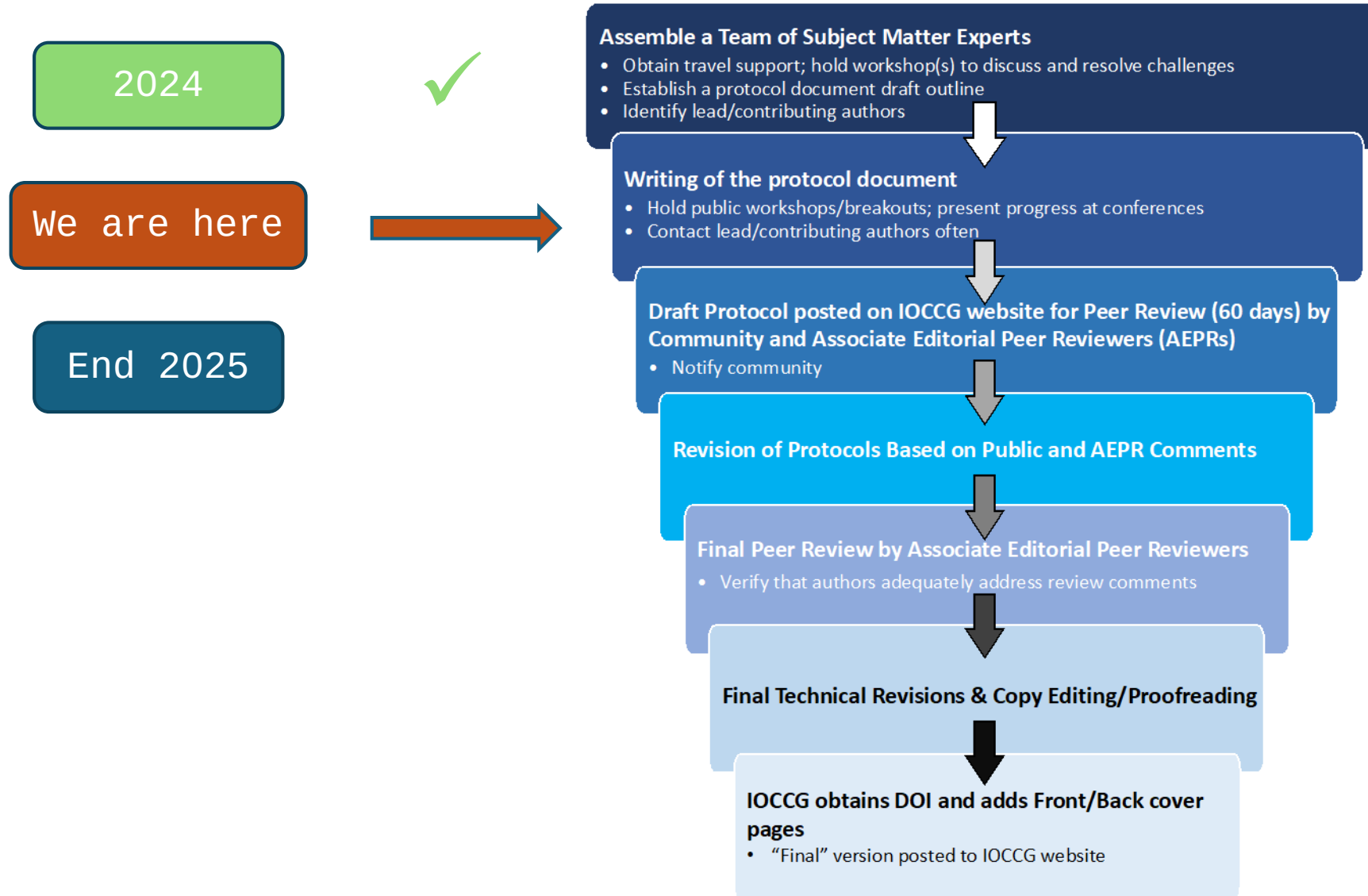


Elisabetta Canuti
European Commission, Joint Research Centre (JRC)

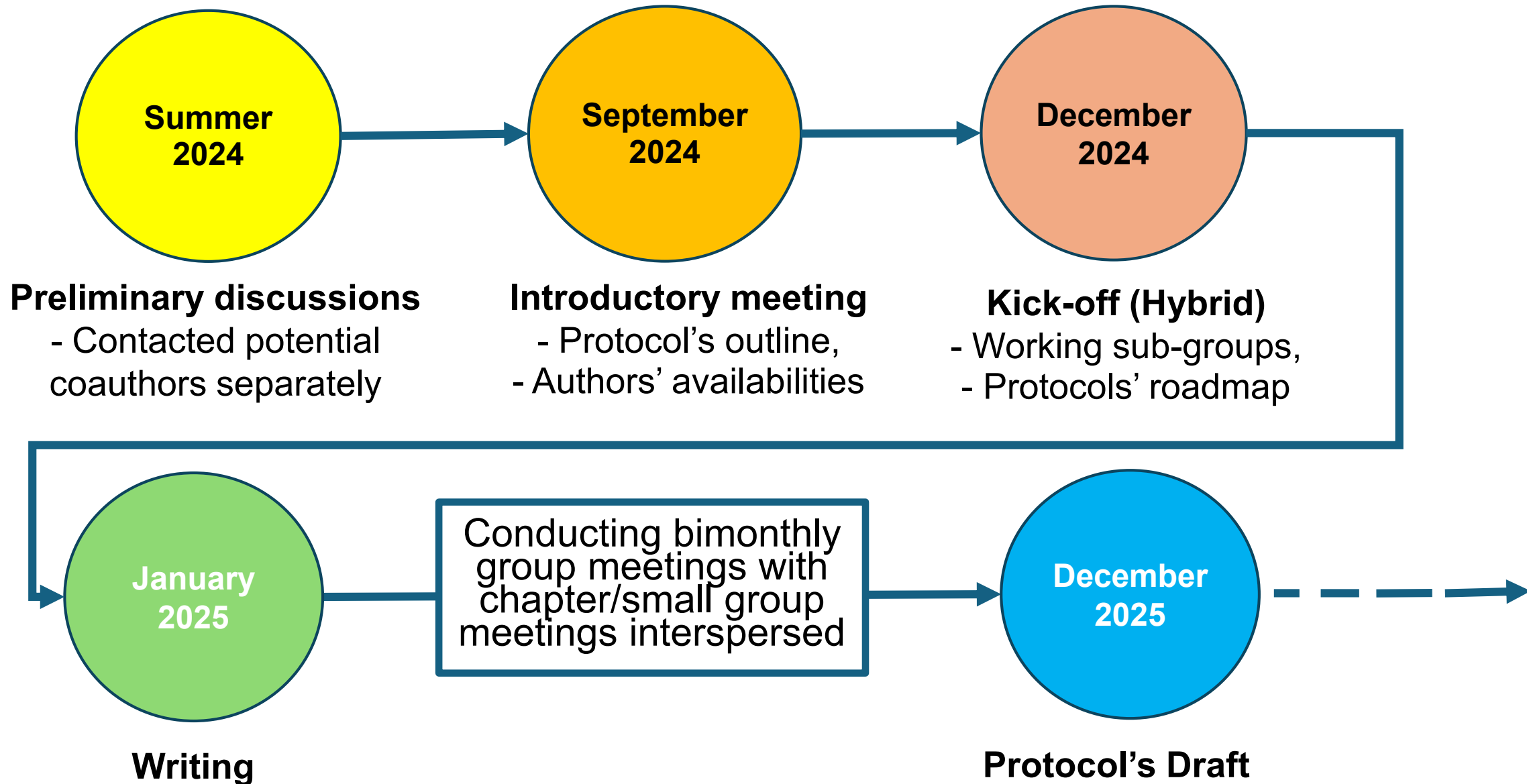


Crystal Thomas
NASA-Ocean Ecology Lab

Process for Measurement Protocols



HPLC Protocol-Timeline



HPLC Subject Matter Experts

Name	Affiliation	Country
Elisabetta Canuti	European Commission, Joint Research Centre (JRC)	Italy
Rafael Mendes	Federal University of Rio Grande (FURG)	Brazil
Ilka Pekeen	Alfred Wegener Institute Centre for Polar and Marine Research (AWI)	Germany
Josephine Ras	Laboratoire d'Océanographie de Villefranche (LOV) and IMEV	France
Louise Schlüter	DHI Laboratory Products	Denmark
Koji Suzuki	Hokkaido University	Japan
Crystal Thomas	Ocean Ecology Laboratory, NASA	United States of America

Potential Reviewers (Not Yet Contacted/Involved)

Lesley Clementson	Commonwealth Scientific and Industrial Research Organisation (CSIRO, retired)	Australia
Suzanne Roy	University of Québec	Canada
Einar Egeland	University of Nordland	Norway

HPLC Protocol-Chapter Outline

Introduction > *Canuti & Thomas*

1. Sampling Procedures > *Ras*

2. Sample Preservation > *Thomas*

3. Sample Extraction > *Pekeen/Mendes*

4. LC Methodology > *Suzuki*

5. Quality Assurance > *Canuti*

6. Additional Considerations > *Thomas*

HPLC Protocol outline

- Introduction (*Elisabetta and Crystal*)
- 1. Sampling procedures- *Josephine, Raphael, Elisabetta, Ika*
 - a. Different water types, regions, seasons, environmental conditions (Ice, inland, coastal, open ocean) CASE I, CASE II, CASE III
 - b. Filter types *Ika*
 - c. Filtration-positive, negative *Josephine*
 - d. Filtration volume *Josephine*
 - e. Storage mechanism (foil, cryovial, histoprep)
- 2. Sample preservation -*Crystal, Ika, Josephine*
 - a. Flash freezing
 - b. Storage temperatures-*Josephine, small experiment?*
 - c. Record keeping
- 3. Extraction - *Raphael, Koji, Ika, Josephine*
 - a. Ext immediately and store v. storage of samples
 - b. Solvent choice *Josephine*
 - i. Volume
 - ii. Internal standards
 - 1. Deciding how to add it
 - 2. ISTDs commonly used
 - c. Filter disruption method
 - d. Filter clarification
 - e. Recoverability
 - f. Stability
 - g. Example procedure
- 4. LC methodology -*Koji, Josephine, Raphael, Elisabetta, Ika, Louise*
 - a. HPLC/UHPLC components
 - i. System overview (eluent, pumps, sample injector, column oven, column, detectors) -> illustration or photo
 - ii. Pumps (for HPLC/UHPLC; high/low-pressure systems)
 - iii. Sample injector (autosampler with temperature controller, sample loop, mixer)
 - iv. Type of columns (with properties)
 - iv-(1). HPLC column (C8, C18(ODS), C18-Pentafluorophenyl, C16-Amide, C30, particle size, length) with mobile phase and column temperature -> a table with references
 - iv-(2). UHPLC column (C8, C18, particle size, length) with mobile phase and column temperature -> a table with references
 - v. Detectors (DAD (UV/Vis), fluorescence)
 - b. Standards and Calibration *Louise*
 - i. Purchased standards
 - ii. Isolating, creating your own standards
 - iii. Calibration with standards
 - ii. Common methods for the calval of remote sensing (features and advantages/challenges of each)
- 5. Quality assurance, including method validation and quality control *Elisabetta, Louise, Crystal*
 - h. How to write a SOP? *Louise*
 - i. LOD/LOQ and metrics (recap of ISO guidelines) *Crystal*
 - j. Validation and comparison *Elisabetta, Crystal*
 - k. Method accreditation *Louise, Elisabetta*
 - l. Reporting considerations (sample reception, reporting results) (template) *Crystal, Louise*
- 6. Other considerations *Crystal*
 - m. LC-MS, uHPLC-MS what it can and can't do *Elisabetta, Koji*
 - n. Application (CHEMTAX, PFTs, SOM, ...) *Koji, Elisabetta, Ika*
- 6. fluo and spectro vs HPLC *Crystal*

ANNEXES:

- SOPs (i. e., IS preparation, full method as for accreditation...);
- List of extinction coefficients (most common, in most common solvents with a link to the main references);