**Workshop on real time quantitative PCR to develop climate services for monitoring harmful algal blooms in their marine environment**

21th to 25th October 2019 at Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research, Am Handelshafen 12, 27570 Bremerhaven, Germany

This workshop is aimed at giving participants an understanding of real time quantitative PCR and its applications in harmful algal bloom (HAB) monitoring. The course includes presentations, discussions, and hands-on demonstrations and covers all aspects of qPCR development and application, from designing a qPCR assay and preparation of defined samples to data analysis and trouble shooting. SYBR green and TaqMan assays will be applied with focus on key species groups of HAB microalgae (e.g., *Alexandrium*, *Ostreopsis*, *Phaeocystis* and *Pseudo-nitzschia* spp.). After completing the course participants will be able to design qPCR assays specific for their individual needs and perform qPCR experiments as well as to interpret and analyze the gained data. Furthermore, the workshop will give insights into fluorescence *in situ* hybridization (FISH) as a validation tool for quantitative real time PCR.

The workshop is intended for persons working in or planning to initiate harmful algal monitoring programs, and are interested in and equipped to applying this molecular technology. Participation is limited to 10 participants, with a documented professional interest in phytoplankton identification by molecular biological methods. The organization team consists of Kerstin Toebe (AWI, Germany), Raffaele Siano (Ifremer, France), Allan D. Cembella (AWI, Germany) and Uwe John (AWI, Germany) . Applications should be sent by email to: Kerstin Tobe (Kerstin.Toebe@awi.de; cc: Allan.Cembella@awi.de, Uwe.John@awi.de, Raffaele.Siano@ifremer.fr) before April 15, 2019. A short letter justifying the participation of the applicant and details of the proposed application of the knowledge gained to HAB monitoring should be submitted. A brief CV of the applicant’s experience and qualifications must also be provided. The course will be taught in English and a good knowledge of English is therefore required. There will be no registration fee, but participants will have to provide for their own travel and accommodation expenses.

The workshop is sponsored by the ERA4CS, CoClime projet (more information on : <https://www.coclime.eu/>) (Grant 690462).

**Goals of the Workshop**

* To give participants a deeper understanding of qPCR and its applications
* To improve harmful algal bloom detection by using molecular tools
* To explore the potential application of qPCR for the monitoring and forecasting of harmful algal blooms in (own) monitoring programs
* To provide guidance for developing own qPCR assays for desired target species
* To validate qPCR achieved results by applying light microscopy and fluorescence *in situ* hybridization (FISH) on selected samples

**Workshop agenda**

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| **1st day** **Monday, 21October 2019** |  |  |
| 9:00- 9:10 | Welcome and Workshop Overview  | Uwe John, Kerstin Toebe Raffaele Siano, Allan Cembella |
| 9:10-9:30 | Presentation of the hosting institute and introduction of workshop attendees  | Uwe John |
| 9:30 - 10:00Theoretical session  | Introduction to project CoCliMEHarmful algal blooms, and coastal ecosystem status indicators in the project CoCliME  | Kerstin Toebe |
| 10:00-10:30 Theoretical session | Introduction to PCR: a general overview* Qualitative PCR and quantitative PCR, theory and applications
* qPCR platforms, amplification and detection

Detection chemistries | Uwe John |
| 10:30-10:45 | Recreational break |  |
| 10:45-11:15 Theoretical session | Different sampling methods of field samplesQuantitative PCR applications in detecting harmful microalgae and their use in HAB monitoring programs with focus on the HAB species involved in monitoring programs of CoCliME endusers  | Raffaele Siano, Kerstin Toebe |
| 11:15-12:00 Theoretical session  | Primer and probe design for qPCR applicationsGeneral overview: * Probes and dyes
* What does primer design effect?
* What are primer dimer formations? How could we minimize it?
* Design and specificity testing of primer and probes
 | Uwe John |
| 12:00-13:00  | Lunch at AWI canteen |  |
| 13:00-17:00 Practical session | * Microscopic counts of cells
* Extraction of DNA of different cell numbers, extraction of DNA from (own) field samples
* Qualitative PCR, preparation of qPCR samples and preparation of standard dilution series
 | Kerstin Toebe, Uwe John, Raffaele Siano |
| 17:00 | End of Workshop day 1 |  |
| **2nd day****Tuesday, 22nd October 2019** |  |  |
| 9:00 – 09:30 Theoretical session | * Creation of standard curves with genomic DNA or plasmid DNA templates
* Standard curve quantification in qPCR approaches:
* absolute and relative quantification
* Inhibition in biological samples and how to compensate
 | Uwe John |
| 9:30-9:45 | Recreational break |  |
| 9:45-12:00 Practical session | * Demonstration of qPCR instrument and qPCR software
* Demonstration of setting up a qPCR experiment
* Setting up a qPCR experiment by participants:

Preparation of standard curves and samples, preparation of positive and negative controls | Kerstin Toebe, Uwe John |
| 12:00-13:00  | * Lunch at AWI canteen
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| 13:00-14:30 Combined theoretical and practical session | Data analysis* How does Step one plus software process the data?
* How are melt curve used?
* How do we use standard curves for quantification?
	+ Quantification methods and equations
	+ Differences in quantification strategies
	+ Advantages and disadvantages of methods
	+ Which effect will assay efficiency have on quantification?
 | Kerstin Toebe, Uwe John |
| 14:30-17:00 Practical session | Analysis of performed qPCR experiments | Kerstin Toebe , Uwe John, Raffaele Siano,  |
| 17:00  | End of Workshop day 2 |  |
| **3rd day****Wednesday, 23October 2019** |  |  |
| 9:00 – 12:00 Practical session  | qPCR analysis of field samples using SYBR Green or TaqMan assays | Kerstin Toebe, Uwe John, Raffaele Siano |
| 12:00-13:00  | Lunch at AWI canteen |  |
| 13:00-17:00 Practical session | qPCR analysis of field samples using SYBR Green and TaqMan approaches, analysis of performed qPCR experiments  |  |
| 17:00  | End of Workshop day 3 |  |
| **4th day** **Thursday, 24 October 2019** |  |  |
| 9:00-9:15 Theoretical session  | Introduction to alternative analysis methods in detecting harmful algal blooms with focus on fluorescence *in situ* hybridization techniques | Kerstin Toebe |
| 9:15-12:00 Practical session | Practical demonstration of FISH experiments and analysis of data | Kerstin Toebe, Uwe John |
| 12:00-13:00  | Lunch at AWI canteen |  |
| 13:00-17:00Combined theoretical and practical session | Further qPCR analysis of field samples, analysis of performed qPCR experiments | Kerstin Toebe, Uwe John, Raffaele Siano, |
| 17:00  | End of Workshop day 4 |  |
| **5th day** **Friday, 25 October 2019** |  |  |
| 9.30-12:00 Combined theoretical and practical session | Ongoing analysis of performed qPCR experiments, optimization strategies,trouble shooting* Future plans, questions and answers
* Workshop summary
 | Kerstin Toebe, Uwe John, Raffaele Siano |
| 12:30 | End of workshop |  |

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